

## Drug Discovery for Psychiatric Disorders

## RSC Drug Discovery Series

### *Editor-in-Chief:*

Professor David Thurston, *London School of Pharmacy, UK*

### *Series Editors:*

Dr David Fox, *Pfizer Global Research and Development, Sandwich, UK*

Professor Salvatore Guccione, *University of Catania, Italy*

Professor Ana Martinez, *Instituto de Quimica Medica-CSIC, Spain*

Professor David Rotella, *Montclair State University, USA*

### *Advisor to the Board:*

Professor Robin Ganellin, *University College London, UK*

### *Titles in the Series:*

- |   |   |
|---|---|
| 1: Metabolism, Pharmacokinetics and Toxicity of Functional Groups | 15: Biomedical Imaging  |
| 2: Emerging Drugs and Targets for Alzheimer's Disease; Volume 1   | 16: Pharmaceutical Salts and Cocrystals                               |
| 3: Emerging Drugs and Targets for Alzheimer's Disease; Volume 2   | 17: Polyamine Drug Discovery  |
| 4: Accounts in Drug Discovery                                     | 18: Proteinases as Drug Targets                                       |
| 5: New Frontiers in Chemical Biology                              | 19: Kinase Drug Discovery   |
| 6: Animal Models for Neurodegenerative Disease                    | 20: Drug Design Strategies: Computational Techniques and Applications |
| 7: Neurodegeneration  | 21: Designing Multi-Target Drugs                                      |
| 8: G Protein-Coupled Receptors                                    | 22: Nanostructured Biomaterials for Overcoming Biological Barriers    |
| 9: Pharmaceutical Process Development                             | 23: Physico-Chemical and Computational Approaches to Drug Discovery   |
| 10: Extracellular and Intracellular Signaling                     | 24: Biomarkers for Traumatic Brain Injury                             |
| 11: New Synthetic Technologies in Medicinal Chemistry             | 25: Drug Discovery from Natural Products                              |
| 12: New Horizons in Predictive Toxicology                         | 26: Anti-Inflammatory Drug Discovery                                  |
| 13: Drug Design Strategies: Quantitative Approaches               | 27: New Therapeutic Strategies for Type 2 Diabetes: Small Molecules   |
| 14: Neglected Diseases and Drug Discovery                         | 28: Drug Discovery for Psychiatric Disorders                          |

### *How to obtain future titles on publication:*

A standing order plan is available for this series. A standing order will bring delivery of each new volume immediately on publication.

### *For further information please contact:*

Book Sales Department, Royal Society of Chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge, CB4 0WF, UK

Telephone: +44 (0)1223 420066, Fax: +44 (0)1223 420247,

Email: [booksales@rsc.org](mailto:booksales@rsc.org)

Visit our website at <http://www.rsc.org/Shop/Books/>

# ***Drug Discovery for Psychiatric Disorders***

Edited by

**Zoran Rankovic**

*Neuroscience, DCR&T, Eli Lilly and Company, Indianapolis, IN 4628, USA*

*Email: rankovic\_zoran@lilly.com*

**Matilda Bingham**

*MSD, Newhouse, Motherwell, ML1 5SH, UK*

*Email: matilda.bingham@virginmedia.com*

**Eric J. Nestler**

*Mount Sinai School of Medicine, New York, New York 10029-5656, USA*

*Email: eric.nestler@mssm.edu*

**Richard Hargreaves**

*Neuroscience Merck, West Point, PA 19486, USA*

*Email: richard\_hargreaves@merck.com*

**RSC Publishing**

RSC Drug Discovery Series No. 28

ISBN: 978-1-84973-365-6

ISSN: 2041-3203

A catalogue record for this book is available from the British Library

© The Royal Society of Chemistry 2012

*All rights reserved*

*Apart from fair dealing for the purposes of research for non-commercial purposes or for private study, criticism or review, as permitted under the Copyright, Designs and Patents Act 1988 and the Copyright and Related Rights Regulations 2003, this publication may not be reproduced, stored or transmitted, in any form or by any means, without the prior permission in writing of The Royal Society of Chemistry or the copyright owner, or in the case of reproduction in accordance with the terms of licences issued by the Copyright Licensing Agency in the UK, or in accordance with the terms of the licences issued by the appropriate Reproduction Rights Organization outside the UK. Enquiries concerning reproduction outside the terms stated here should be sent to The Royal Society of Chemistry at the address printed on this page.*

The RSC is not responsible for individual opinions expressed in this work.

Published by The Royal Society of Chemistry,  
Thomas Graham House, Science Park, Milton Road,  
Cambridge CB4 0WF, UK

Registered Charity Number 207890

For further information see our web site at [www.rsc.org](http://www.rsc.org)

Printed in the United Kingdom by Henry Ling Limited, at the Dorset Press, Dorchester,  
DT1 1HD



# *Preface*

The discovery and development of medicines for the treatment of psychiatric disorders such as schizophrenia and depression has been an important medical advance in the 20<sup>th</sup> century. These drugs have given benefit through the treatment of psychiatric patients as well as providing the scientific basis for understanding the underlying pathophysiology of many mental illnesses. However, despite quite remarkable progress, psychiatric disorders are still the leading cause of disability worldwide with urgent medical need for novel, more effective, and safer treatments.

High attrition rates have characterized the development of new drugs for psychiatric disorders over the past two decades. Consequently, only a few new drugs were approved in this period and many, although with improved some aspects of tolerability and safety, are based on mechanisms introduced decades ago. The high cost and low probability of success in developing psychiatric medicines has, worryingly, driven a number of pharmaceutical companies out of the field. The challenge for scientists working on the psychiatric disorders has therefore never been greater. It is now more than ever critical to learn from the past, exploit and advance the current understanding of the psychiatric disorders and apply the learning to increase the effectiveness of drug discovery and development. In this respect, it is our sincere hope that the students and scientists in this discipline will find this volume on “Drug Discovery for Psychiatric Disorders” helpful.

Written by top experts from both academia and industry, this book covers the history, current state of the art, challenges, opportunities and future directions in areas such as pathophysiology, medicinal chemistry, and drug discovery for major psychiatric disorders. In the book-opening chapter David Michelson and Kathryn M. Connor provide an overview of the major psychiatric disorders from the clinical perspective, outlining the symptoms, classification, diagnosis, prognosis and prevalence, as well as current treatments

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

and medical needs. The remaining twenty chapters are divided into four sections: 1) Schizophrenia, 2) Anxiety and Depression, 3) Other psychiatric disorders, and 4) Challenges and future directions in drug discovery for psychiatric disorders.

The section focusing on schizophrenia is introduced by Armin Szegedi and Michael Egan (Chapter 2) who describe how our understanding of biological processes involved in schizophrenia has evolved over the past 60 years, from the seminal dopamine hyperfunction to a more recent glutamate hypofunction hypothesis of the disease pathophysiology. The authors conclude the chapter with an overview of potential novel targets identified from the gene association studies.

All currently prescribed drugs for the treatment of schizophrenia derive from the dopamine hypothesis. For many years it was a common view, based on experience from the first generation of antipsychotics, that antipsychotic efficacy is intrinsically associated with adverse effects such as extrapyramidal side effects (EPS) and prolactin release, since they are all mediated by antagonism of the same dopamine D<sub>2</sub> receptor. Extensive research efforts towards antipsychotics with an improved safety profile led to the discovery and development of the second generation (atypical) antipsychotics such as clozapine, and more recently the third generation typified by D<sub>2</sub> receptor partial agonist aripiprazole. In chapter 3, David P. Rotella describes the evolution of the dopaminergic antipsychotic drugs, as well as some of more recent approaches that engage other monoaminergic receptors in order to design novel agents with a wider spectrum of efficacy and more favorable safety and tolerability profile. In the following chapter John A. Morrow, Robert Gilfillan and Stuart A. Neale (Chapter 4) focus on the emerging glutamate hypothesis of schizophrenia born from an observation that NMDA receptor antagonists such as ketamine and PCP induce schizophrenia-like symptoms in normal individuals and exacerbate positive, negative and cognitive symptoms in patients with schizophrenia. Since direct NMDA receptor agonism is associated with seizure and neurotoxicity, a number of indirect activation approaches have been pursued across the industry, many of which are described in this chapter. Whilst the existing antipsychotic drugs effectively treat positive and to some extent negative symptoms, they do not provide adequate improvement in cognitive deficits in schizophrenia (CDS). Untreated CDS results in poor work, social and independent living outcomes. In chapter 5 Simon Ward describes a research project pursued at GlaxoSmithKline towards novel AMPA receptor positive allosteric modulators for the treatment of CDS. This is followed by Bruce N. Rogers and David L. Gray wider review of the field, including most advanced approaches and drug discovery efforts for the treatment of CDS (Chapter 6).

In the introductory chapter for the section on depression and anxiety disorders (Chapter 7), Georgia E. Hodes and Scott J. Russo review *in vivo* models used to understand the disease underlying neurobiology, the currently prevailing monoamine-based hypothesis of depression, and discuss the most promising novel drug discovery approaches. Despite a number of mechanistically distinct classes of antidepressants that are currently available, including

monoamine oxidase inhibitors (MOIs), tricyclic antidepressants (TCAs) and the currently predominant Selective Serotonin Reuptake Inhibitors (SSRIs), more than a third of the patient population are still resistant to treatment. Even in the patient population that responds well, the onset of action is very slow at typically 4–6 weeks. These deficiencies provided a major drive across the pharmaceutical industry to look beyond the SSRI approach, as discussed in great detail by Peter Gallagher in Chapter 8.

Anxiety and fear are universal human emotions that serve an essential role in shaping adaptive behavior. However, excessive and sustained levels of anxiety may lead to marked suffering and disability. In fact, anxiety disorders are among the most common and disabling psychiatric illnesses. James W. Murrough, Daniela Schiller, and Dennis S. Charney review in Chapter 9 the epidemiology, clinical features, current and potential novel therapeutic strategies for anxiety disorders with a particular focus on panic disorder and posttraumatic stress disorder (PTSD).

Mood disorders, including both major depressive (MDD) and bipolar disorders (BPD), are forms of chronic mental illness associated with alterations in normal affective response that result in profound disruptions in daily living and for which there are limited treatment options. Recent preclinical and clinical findings led to a major shift in drug discovery for mood disorders from a sole focus on the traditional monoamine-based approaches to the modulation of glutamatergic signaling. Much of this work is still in the discovery phase and Carrie K. Jones, P. Jeffrey Conn and Craig W. Lindsley in Chapter 10 describe some of the emerging Medicinal Chemistry and Drug Discovery in this area.

Another alternative to the traditional monoamine approaches for the treatment of mood disorders is to target the hypothalamic-pituitary-adrenal (HPA) axis. Dysregulation of the HPA axis, the fundamental stress regulator in mammals, has long been implicated in playing a critical role in the pathogenesis of mood disorders, and more specifically major depression. The current understanding of how molecular targets within the HPA axis can be used as modulation points for novel therapeutics to treat mood disorders and anxiety is discussed by Charles B. Nemeroff in Chapter 11. This area of drug discovery in particular has highlighted the challenges associated with obtaining data from preclinical models which can usefully predict efficacy in the clinic. Many of the standard preclinical models employed in this therapeutic area are based on the monoamine hypothesis and hence are not directly applicable to drugs with a new mode of action such as those targeting the HPA axis. In Chapter 12 Shigeyuki Chaki and Kosuke Kanuma revisit some of these targets from the Medicinal Chemistry perspective and broaden the discussion to the role of neuropeptides in modulation of the HPA axis and reward pathways. The authors highlight some of the targets under active investigation for the treatment of mood disorders and discuss the drug chemotypes and the associated medicinal chemistry challenges.

The next section of the book covers neuropathophysiology and main drug discovery approaches for other psychiatric disorders: bipolar disorder (Chapter 13 by Moghis U. Ahmad, Shoukath M. Ali and Saifuddin Sheikh); drug

addiction (Chapter 14 by C.D. Gipson, Peter W. Kalivas); autism (Chapter 15 by Paul Wang, Rebecca Hammond, Friso Postma and Aileen Healy); and insomnia (Chapter 16 by Christopher J. Winrow, Anthony L. Gotter, Paul J. Coleman, Richard Hargreaves and John J. Renger).

The final section of this volume focuses on the main challenges and potential future directions in drug discovery for psychiatric disorders. A prerequisite for efficacy of psychiatric drugs is the ability to cross the blood-brain-barrier (BBB) and reach the site of action in the central nervous system (CNS). In Chapter 17 James A. Baker and Iain J. Martin give an overview of *in vitro* and *in vivo* methods commonly used in the pharmaceutical industry for predicting and measuring CNS exposure. In chapter 18 Matilda Bingham and Zoran Rankovic discuss interpretation and application of data derived from these methods and physicochemical properties in prospective design of molecules with optimal CNS exposure and safety profile.

Modulation of multiple targets within relevant biological pathways and networks is increasingly been recognized as a superior approach towards the next generation of treatments for diseases with complex, polygenic aetiology such as psychiatric disorders. Consequently, there is an ever increasing interest in deliberate and rational design of ligands that act selectively on specific multiple targets (multi target drug discovery). In Chapter 19 Zoran Rankovic and Richard Morphy discuss medicinal chemistry strategies and challenges in applying this rapidly emerging paradigm in drug discovery for the treatment of psychiatric disorders. In the search for novel psychiatric treatments, many compounds that have shown promising pharmacological properties in disease models have failed to produce benefit in patients. In fact, poor predictive power of the existing animal models is often quoted as one of the main reasons for high attrition rates in development of CNS targeting medicines. Some of the factors leading to erroneous decision-making based on animal model data and potential improvements are discussed by Mark D. Tricklebank and Joseph P. Garner in Chapter 20. The challenge of drug discovery for psychiatric diseases is further compounded by the paucity of processes and technologies that can provide a true translational bridge between preclinical studies and subsequent clinical testing and evaluation. Similarly, identifying an optimal dosing regime that would provide required CNS exposure is critical, since failure to achieve adequate target occupancy would inevitably increase the risk of failure in the clinic due to lack of efficacy. Richard J. Hargreaves and Eugenii A. Rabiner describe in Chapter 21 translational strategies, methods and techniques that have been developed and implemented over the recent years to address these challenges, such as PET, SPECT and fMRI imaging techniques. The volume is concluded with discussion of the future challenges and directions in drug discovery for psychiatric disorders by Darryle D. Schoepp and Richard J. Hargreaves.

We are very grateful to the many contributors to this volume for their insightful chapters and timely delivery. We hope that this book succinctly captures the beauty, complexity, diversity and importance of the fundamental research and drug discovery for psychiatric disorders. Given the wealth of

knowledge and huge unmet medical need, it is an absolute imperative that the pharmaceutical industry, together with academia and governments remain committed to sustained advancement of the research and development of innovative treatments for psychiatric disorders.

Dr. Zoran Rankovic, Eli Lilly, Indianapolis, USA  
Dr. Matilda Bingham, Redx Oncology, Liverpool, UK  
Prof. Eric J. Nestler, Mount Sinai School of Medicine,  
New York, NY, US  
Dr. Richard Hargreaves, Merck, West Point, PA, US



# Contents

<b>Chapter 1</b>	<b>Psychiatric Disorders – an Overview</b>	<b>1</b>
	<i>Kathryn M. Connor and David Michelson</i>	
1.1	Introduction	1
1.2	Diagnostic Considerations	2
1.3	Treatment Options	4
1.3.1	Schizophrenia	4
1.3.2	Major Depression	4
1.3.3	Anxiety Disorders	5
1.3.4	Insomnia	6
1.3.5	Bipolar Disorder	6
1.3.6	Other Psychiatric Disorders	6
1.4	Unmet Medical Needs	7
	References	9
 <b>Section 1: Schizophrenia</b>		
<b>Chapter 2</b>	<b>The Pathophysiology of Schizophrenia</b>	<b>13</b>
	<i>Armin Szegedi and Michael Egan</i>	
2.1	History of Clinical Concepts	13
2.2	Epidemiology, Longitudinal Course and Outcome	15
2.3	Etiological Concepts for Schizophrenia	17
2.4	Treatment of Schizophrenia	18
2.5	Neurochemical Abnormalities	19
2.5.1	Dopamine	20
2.5.2	Glutamate	22
2.5.3	GABA	23
2.5.4	Serotonin	24
2.6	Neuropathology	24

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

2.7	Brain Imaging and Electrophysiological Abnormalities	25
2.8	Genetics	28
2.8.1	Heritability of Schizophrenia	28
2.8.2	Results of Linkage Studies	29
2.8.3	Results of Association Studies	30
2.9	Integrated Biological Theories	31
2.10	Conclusions	32
	References	32
<b>Chapter 3</b>	<b>Monoaminergic Approaches for Treatment of Schizophrenia</b>	<b>35</b>
	<i>David P. Rotella</i>	
3.1	Introduction	35
3.1.1	Overview of Antipsychotic Pharmacotherapy	35
3.1.2	Summary of Second-generation Agents	36
3.2	Serotonergic Receptor Targets	39
3.2.1	5-HT <sub>1A</sub> Partial Agonists	39
3.2.2	5-HT <sub>2C</sub> Agonists	40
3.2.3	5-HT <sub>2A</sub> Antagonists	42
3.3	Recent Results in Dopaminergic Receptors	43
3.3.1	D <sub>3</sub> Receptors	43
3.3.2	D <sub>4</sub> Receptors	47
3.4	Selective Poly-target Approaches	48
3.4.1	D <sub>2</sub> /5-HT <sub>1A</sub>	48
3.4.2	D <sub>2</sub> /Serotonin Reuptake Inhibition	49
3.5	Summary and Outlook	51
	References	52
<b>Chapter 4</b>	<b>Glutamatergic Approaches for the Treatment of Schizophrenia</b>	<b>56</b>
	<i>John A. Morrow, Robert Gilfillan and Stuart A. Neale</i>	
4.1	Introduction	56
4.2	Targeting the Co-agonist Site of the NMDA Receptor	58
4.2.1	Glycine Transporter Inhibitors	58
4.2.2	D-serine: a Selective NMDA Receptor Modulator	65
4.3	AMPA Receptor Modulators	69
4.3.1	Preclinical Studies with AMPA Receptor Modulators	71
4.3.2	Clinical Studies with AMPA Receptor Modulators	72



<i>Contents</i>	xiii
4.4 Metabotropic Glutamate Receptors	74
4.4.1 Group II mGlu Receptors	74
4.4.2 Group I mGlu Receptors	78
4.5 Conclusion	81
References	82
<b>Chapter 5 Discovery and Clinical Data for a Novel AMPA Receptor Positive Modulator</b>	<b>99</b>
<i>Simon E. Ward</i>	
5.1 Introduction	99
5.2 Discovery Landscape	103
5.2.1 In-house Screening Methods	104
5.3 Case Study of Discovery Project – Background	107
5.4 Case Study of Discovery Project – Crystallography	107
5.5 Case Study of Discovery Project – Hit to Lead Optimization	109
5.6 Crystallographic Studies	114
5.7 Characterization of Preclinical Development Candidate	116
5.8 Clinical Characterization of Development Molecule	119
5.9 Conclusions and Future Perspectives	119
Acknowledgements	120
References	120
<b>Chapter 6 Treating the Cognitive Deficits of Schizophrenia</b>	<b>123</b>
<i>Bruce N. Rogers and David L. Gray</i>	
6.1 Introduction: Biological Rationale for Treating the Cognitive Deficits in Schizophrenia	123
6.2 Alpha 7 Nicotinic Acetylcholine Receptors	124
6.2.1 Alpha 7 Nicotinic Acetylcholine Receptor Agonists	124
6.2.2 Alpha 7 Nicotinic Acetylcholine Receptor Positive Allosteric Modulators	129
6.3 Dopamine D <sub>1</sub> Receptor Agonists	132
6.4 Additional Mechanisms of Significant Interest in the Treatment of Cognitive Deficits in Schizophrenia	137
6.4.1 Muscarinic M <sub>1</sub> Positive Allosteric Modulators	137
6.4.2 Muscarinic M <sub>1</sub> Allosteric Agonists	139
6.4.3 Serotonin Subtype 6 (5-HT <sub>6</sub> ) Receptor Antagonists	140
6.4.4 Histamine H3 Receptor Antagonists	142

6.4.5	$\gamma$ -Aminobutyric Acid A Receptor Subtype Selective Agonists (GABA <sub>A</sub> )	143
6.4.6	Catechol- <i>O</i> -methyl Transferase Inhibitors (COMT)	144
	References	144

## Section 2: Depression and Anxiety

<b>Chapter 7</b>	<b>The Neurobiology of Depression and Anxiety: How Do We Change from Models of Drug Efficacy to Understanding Mood and Anxiety Disorders?</b>	<b>159</b>
	<i>Georgia E. Hodes and Scott J. Russo</i>	
7.1	Introduction	159
7.2	A Brief History of the Development of Current Antidepressant Treatments	160
7.2.1	Monoamines and Selective Serotonin Reuptake Inhibitors	161
7.2.2	Catecholamines and Tricyclic Antidepressants	162
7.3	Animal Models of Depression and Anxiety	163
7.3.1	Chronic Stressors	163
7.3.2	Acute Stress	166
7.4	Brain Regions Implicated in Depression and Anxiety Disorders	167
7.4.1	Hypothalamus	168
7.4.2	Hippocampus	169
7.4.3	Prefrontal Cortex	170
7.4.4	Nucleus Accumbens	171
7.4.5	Amygdala	171
7.5	New Targets for Treatment of Anxiety and Mood Disorders	173
7.5.1	Ketamine	173
7.5.2	Cytokines	174
7.5.3	Epigenetic Regulators of Depression	175
7.6	Conclusion: Creating Personalized Treatments for Anxiety and Depression	175
	References	176
<b>Chapter 8</b>	<b>Beyond SSRIs: Second-generation Reuptake Inhibitors for the Treatment of Depression</b>	<b>184</b>
	<i>Peter T. Gallagher</i>	
8.1	Profile and Limitations of Selective Serotonin Reuptake Inhibitors (SSRIs)	184

8.2	Serotonin Reuptake Inhibition Augmentation Strategies	186
8.2.1	Serotonin Reuptake Inhibition and 5-HT <sub>1A</sub> Antagonism	186
8.2.2	Serotonin Reuptake Inhibition and 5-HT <sub>1A</sub> Agonism	189
8.2.3	Serotonin Reuptake Inhibition and 5-HT <sub>1D</sub> Antagonism	191
8.2.4	Serotonin Reuptake Inhibition and 5-HT <sub>2A</sub> Antagonism	192
8.2.5	Serotonin Reuptake Inhibition and 5-HT <sub>3</sub> Antagonism	195
8.2.6	Serotonin Reuptake Inhibition and Non-serotonergic Receptor Interaction	195
8.2.7	Serotonin and Norepinephrine Reuptake Inhibition	196
8.2.8	Serotonin, Norepinephrine and Dopamine Reuptake Inhibition	199
8.3	Pre- and Post-synaptic 5-HT Targets	205
8.3.1	5-HT <sub>1A</sub> Agonism	205
8.3.2	5-HT <sub>1A</sub> Antagonists	208
8.3.3	5-HT <sub>2C</sub> Agonists and Antagonists	210
	References	212

## **Chapter 9 Neurocircuitry of Anxiety Disorders: Focus on Panic Disorder and Post-traumatic Stress Disorder** **226**

*James W. Murrough, Daniela Schiller and  
Dennis S. Charney*

9.1	Introduction	226
9.2	Epidemiology and Clinical Features of Anxiety Disorders	227
9.2.1	Epidemiology of Anxiety Disorders	227
9.2.2	Clinical Features of Panic Disorder	228
9.2.3	Clinical Features of PTSD	228
9.3	Advances in Neurocircuitry Models of Fear	229
9.3.1	Overview of Fear Conditioning	229
9.3.2	The Essential Role of the Amygdala in Fear Conditioning	229
9.3.3	The Essential Role of the Prefrontal Cortex in Fear Extinction	231
9.4	Neurocircuitry of Panic Disorder	233
9.4.1	Preclinical Models	233
9.4.2	Human Behavioural and Pharmacological Challenge Studies	235

9.4.3	Human Neuroimaging Studies	236
9.4.4	Genetics Studies	238
9.5	Neurocircuitry of PTSD	239
9.5.1	Preclinical Models	239
9.5.2	Human Behavioural and Pharmacological Challenge Studies	240
9.5.3	Human Neuroimaging Studies	241
9.5.4	Genetics Studies	243
9.6	Examples of Novel Therapeutic Targets in Anxiety Disorders	244
9.6.1	Glutamate and GABA	244
9.6.2	Neuropeptides	245
9.7	Summary and Conclusion	246
	Conflict of Interest Disclosure	246
	Acknowledgements	246
	References	247
<b>Chapter 10</b>	<b>Glutamate Approaches Towards the Treatment of Mood Disorders</b>	<b>258</b>
	<i>Carrie K. Jones, P. Jeffrey Conn and Craig W. Lindsley</i>	
10.1	Introduction to Mood Disorders	258
10.2	Glutamatergic Neurotransmission	261
10.3	Glutamatergic Targets for the Treatment of Mood Disorders	262
10.3.1	Inhibitor of Glutamate Release	263
10.3.2	Glial Glutamate Transporter Enhancers	264
10.3.3	NMDA Antagonists	265
10.3.4	AMPA Potentiators	266
10.3.5	Metabotropic Glutamate Receptors	267
10.4	Conclusion	269
	Acknowledgements	269
	References	269
<b>Chapter 11</b>	<b>Modulation of HPA Axis Function for Treatment of Mood Disorders</b>	<b>276</b>
	<i>Shaun P. Brothers, Claes Wahlestedt and Charles B. Nemeroff</i>	
11.1	The Hypothalamic-Pituitary-Adrenal (HPA) Axis	276
11.2	The HPA Axis in Mood Disorders	278
11.3	Non-mood-related Effects of HPA-Axis Hyperactivity	279

11.4	Modulation of the HPA Axis as a Therapeutic Strategy to Treat Mood Disorders	280
11.5	CRF1 Receptor Antagonists in Mood and Anxiety Disorders	280
11.6	CRF2 Receptor Antagonists in the Treatment of Mood Disorders	283
11.7	CRF Binding Protein	283
11.8	Arginine Vasopressin 1b Receptors	283
11.9	Anti-glucocorticoid Therapy for Mood Disorders	284
11.10	Glucocorticoid and Mineralocorticoid Receptor Activation in the Treatment of Non-psychotic and Anxious Mood Disorders	286
11.11	Neuropeptides and HPA Axis Control of Mood Disorders	287
11.11.1	Substance P	288
11.11.2	Neuropeptide Y	289
11.11.3	Galanin	290
11.12	FKBP5 as a Genetically Linked HPA Axis Therapeutic Target	290
11.13	Conclusions and Expert Opinion	291
	References	293

## **Chapter 12 Neuropeptide Receptors: Novel Therapeutic Targets for Depression and Anxiety Disorders** **300**

*Shigeyuki Chaki and Kosuke Kanuma*

12.1	Introduction	300
12.2	Neuropeptide Receptors that Regulate Activity of the HPA Axis	302
12.2.1	CRF1 Receptor	302
12.2.2	V1b (V3) Receptor	307
12.3	Neuropeptide Receptors that Regulate Reward Activity	310
12.3.1	MCH1 Receptor	311
12.3.2	$\kappa$ Opioid Receptor	314
12.4	Neuropeptide Systems that Regulate Feeding	317
12.4.1	Neuropeptide Y	317
12.4.2	Other Neuropeptide Receptors	319
12.5	Conclusions and Future Direction	322
	References	322

### Section 3: Other Psychiatric Disorders

## Chapter 13 Therapeutic Approaches to Bipolar Disorder 333

*Moghis U. Ahmad, Shoukath M. Ali, Saifuddin Sheikh,  
Ateeq Ahmad and Imran Ahmad*

13.1	Introduction	333
13.2	Causes of Bipolar Disorder	334
13.2.1	Genetic	335
13.2.2	Childhood Precursors	335
13.2.3	Neural Processes	335
13.2.4	Melatonin Activity	336
13.2.5	Psychological Processes	336
13.2.6	Life Events and Experiences	336
13.3	Signs and Symptoms	337
13.3.1	Depressive Episode	337
13.3.2	Manic Episode	337
13.3.3	Hypomanic Episode	338
13.3.4	Mixed Affective Episode	338
13.4	Diagnosis	338
13.5	Treatment of Bipolar Disorder	339
13.5.1	Pharmacotherapy	340
13.5.2	Lithium (Lithium Carbonate)	340
13.5.3	Valproate (Depakote, Depakene)	341
13.5.4	Carbamazepine (Tegretol)	342
13.5.5	Lamotrigine	342
13.5.6	Selective Serotonin Reuptake Inhibitors (SSRIs)	343
13.5.7	Serotonin-norepinephrine Reuptake Inhibitors (SNRI)	344
13.5.8	Noradrenergic and Specific Serotonergic Antidepressants (NaSSAs)	344
13.5.9	Norepinephrine (Noradrenaline) Reuptake Inhibitors (NRIs)	345
13.5.10	Tricyclic Antidepressants (TCAs)	345
13.5.11	Monoamine Oxidase Inhibitors (MAOIs)	345
13.6	New Therapeutic Agents	346
13.6.1	Identification of Novel Molecular Target	347
13.6.2	Protein Kinase C (PKC)	347
13.6.3	PKC Signalling Cascade in Bipolar Disorder	348
13.6.4	PKC inhibitors: Novel Therapeutics for Acute Mania?	349
13.6.5	Omega-3 Fatty Acids in Bipolar Disorder	351
13.7	Promising New Drugs under Clinical Development	351

13.8	Other Treatment Options (Off-label Drugs) for Bipolar Disorder	352
13.8.1	Neurontin (Gabapentin)	352
13.8.2	Topamax (Topiramate)	353
13.8.3	ABS-103	353
13.9	Conclusion	353
	References	353

## **Chapter 14 Pharmacotherapies for Drug Addiction 357**

*C. D. Gipson and P. W. Kalivas*

14.1	Introduction	357
14.2	Dopamine Transporter and D <sub>3</sub> Receptors: Implications for Pharmacotherapy	358
14.2.1	Alcohol	360
14.2.2	Cocaine and Methamphetamine	361
14.3	Substance P and the NK1 Receptor	362
14.3.1	Alcohol	362
14.3.2	Opioid Addiction	363
14.4	Glutamatergic Signalling: Dysregulation in Addiction and Pharmacological Targeting	363
14.4.1	Alcohol	367
14.4.2	Nicotine	368
14.4.3	Cocaine and Heroin	369
14.5	Opioid System as a Potential Pharmacotherapeutic Target	370
14.5.1	Alcohol	370
14.5.2	Nicotine	371
14.5.3	Cocaine, Heroin and Methamphetamine	371
14.6	Other Pharmacotherapies for Drug Dependence	372
14.6.1	Orexin	372
14.6.2	Cannabinoids	372
14.6.3	Replacement Therapies	373
14.6.4	Antidepressants	373
14.6.5	Vaccines	374
14.6.6	Pharmacogenetics	374
14.7	Conclusions	375
	References	376

## **Chapter 15 Emerging Research towards the Understanding and Treatment of Autism 384**

*Paul Wang, Rebecca Hammond, Friso Postma and Aileen Healy*

15.1	Autism and Autism Spectrum Disorders	384
15.1.1	Prevalence of ASD	386

15.2	Etiology of ASD (Defining the Molecular and Neuropathophysiology)	387
15.2.1	Heritable Risk Factors	387
15.2.2	Chromosomal Rearrangements	388
15.2.3	Copy Number Variations	390
15.2.4	Rare Mutations with Known Etiology	391
15.2.5	Environmental Factors	396
15.3	Current Treatments for ASD	396
15.4	Emerging Treatments for ASD	398
15.4.1	The mGluR Theory of FXS	398
15.4.2	mGluR Negative Allosteric Modulators in FXS and ASD	398
15.4.3	GABA <sub>B</sub> Agonists	400
15.4.4	STX209 in FXS and ASD	400
15.4.5	Other Emerging Treatments	401
15.5	Clinical Outcome Measures	402
15.6	Biomarkers	404
15.7	Conclusions	405
	References	405

## **Chapter 16 Recent Chronology of Orexin Pharmacology and Its Potential as a Treatment for Primary Insomnia 416**

*Christopher J. Winrow, Anthony L. Gotter, Paul J. Coleman, Richard Hargreaves and John J. Renger*

16.1	Introduction	416
16.2	Current Understanding of Orexin Biology – From Genetics to Pharmacology	417
16.2.1	Genetic Dissection of Orexin Function	417
16.2.2	Orexin Ligands Promote Wakefulness and Arousal	420
16.2.3	Orexin-mediated Pathways Controlling Sleep Architecture	421
16.2.4	Modulation of Orexin Signalling	424
16.3	Identification of Orexin Receptor Antagonists	425
16.3.1	OX <sub>1</sub> R-selective Antagonism to Probe <i>in vivo</i> Receptor Function	426
16.3.2	Dual Orexin Receptor Antagonists Selectively Attenuate Arousal	427
16.3.3	OX <sub>2</sub> R-selective Antagonism	430
16.4	Orexin Function beyond Sleep	431
16.4.1	Orexin Signalling and Reward Pathways	431
16.4.2	Orexin and Mood	432
16.4.3	Orexigenic Roles	433



16.5	OXR Antagonism Provides a Novel Therapeutic Alternative to Standard of Care for Insomnia	434
	References	436

#### Section 4: Challenges and Future Directions in Drug Discovery for Psychiatric Disorders

### Chapter 17 Crossing the Blood-brain Barrier – Methods for Evaluating CNS Exposure **445**

*James A. Baker and Iain J. Martin*

17.1	Introduction	445
17.1.1	Importance of Assessing CNS Penetration	445
17.1.2	BBB Structure and Function	446
17.1.3	Relationship between CNS Compartments	447
17.2	Methods Commonly Used to Assess CNS Penetration	449
17.2.1	<i>In silico</i>	449
17.2.2	<i>In vitro</i>	449
17.2.3	<i>In vivo</i>	451
17.2.4	Microdialysis	455
17.2.5	Receptor Occupancy	455
17.3	The Serial CSF Approach	456
17.3.1	Experiences of the Method	456
17.3.2	Factors Impacting CSF Data	458
17.4	Strategies for Optimizing CNS Penetration	459
17.5	Preclinical Species as Predictors for Human CNS Penetration	460
17.6	Conclusions	461
	Acknowledgements	462
	References	462

### Chapter 18 Medicinal Chemistry Challenges in CNS Drug Discovery **465**

*Matilda Bingham and Zoran Rankovic*

18.1	Introduction	465
18.2	The Unique Challenge Posed by the Blood-brain Barrier	466
18.2.1	Interpreting CNS Specific DMPK Methods to Facilitate Design of Centrally Acting Medicines	468
18.2.2	Physicochemical Properties for CNS Penetration	472
18.2.3	P-glycoprotein Efflux at the BBB	486
18.2.4	Increasing Systemic Exposure to Increase Brain Exposure	489

18.3	Safety and Tolerability: Common Challenges in CNS Drug Discovery	489
18.3.1	Optimization of hERG Selectivity	491
18.3.2	Phospholipidosis	500
18.4	Summary	501
	References	503
<b>Chapter 19</b>	<b>Multi-target Drug Discovery for Psychiatric Disorders</b>	<b>510</b>
	<i>Zoran Rankovic and Richard Morphy</i>	
19.1	Introduction	510
19.2	Advantages and Disadvantages of FDCs and DMLs	511
19.3	Strategies and Challenges of Designing Multiple Ligands	513
19.3.1	Medicinal Chemistry Strategies for DML Lead Generation	513
19.3.2	Medicinal Chemistry Challenges	516
19.4	Multi-target Drug Discovery for Psychiatric Disorders – Case Studies	522
19.4.1	Depression: SERT-plus Combinations	523
19.4.2	Schizophrenia: Ziprasidone and Other Antipsychotic Agents	527
19.5	Summary and Outlook	530
	References	531
<b>Chapter 20</b>	<b>The Possibilities and Limitations of Animal Models for Psychiatric Disorders</b>	<b>534</b>
	<i>Mark D. Tricklebank and Joseph P. Garner</i>	
20.1	Introduction	534
20.2	The Drug-discovery Pipeline and an Overview of Potential Issues in Animal Research	536
20.2.1	Preclinical Explanations for Lack of Clinical Efficacy	536
20.3	The Need for Disease Models	540
20.4	Validation Approaches	542
20.5	Disease Diagnosis and Animal Models	547
20.6	The Symptom Cluster Approach	548
20.7	Use of Biomarkers	551
20.7.1	The Role of Biomarkers in Model Development and Validation	551
20.7.2	The Role of Biomarkers in Target Assessment and Refinement	552

<i>Contents</i>	xxiii
20.8 Conclusions	553
Acknowledgements	554
References	554
<b>Chapter 21 Translational PET Imaging Research in Psychiatry</b>	<b>558</b>
<i>Richard J. Hargreaves and Eugenii A. Rabiner</i>	
21.1 Introduction	558
21.2 Accelerating Proof-of-concept Testing	559
21.3 PET Study Designs for CNS Drug Discovery	560
21.3.1 Biodistribution Imaging	560
21.3.2 PET Target Engagement Imaging	562
21.3.3 Pharmacodynamic Imaging	563
21.4 Examples of PET Imaging in CNS Drug Discovery	564
21.5 PET Imaging in the Treatment and Understanding of Psychiatric Disease	565
21.5.1 Schizophrenia	565
21.5.2 Depression	566
21.6 Challenges to Using Molecular Imaging in Drug Development	567
21.7 Summary and Outlook	568
References	568
<b>Chapter 22 Future Drug Discovery for Psychiatric Disorders</b>	<b>574</b>
<i>Darryle D. Schoepp and Richard J. Hargreaves</i>	
<b>Subject Index</b>	<b>579</b>



## CHAPTER 1

# *Psychiatric Disorders – an Overview*

KATHRYN M. CONNOR AND DAVID MICHELSON\*

Merck Research Laboratories, UG4C-018, Box 1000, North Wales,  
PA 19454, USA

\*Email: David\_michelson@merck.com

## 1.1 Introduction

The psychiatric illnesses represent, collectively, a group of brain disorders characterized by behavioural and cognitive abnormalities and dysfunction. That psychiatric disorders exist and are abnormal states has been recognized throughout history, though, as with the history of illness generally, the meanings attributed to them have varied considerably across cultures and eras. Today psychiatric disorders are understood as behavioural and cognitive syndromes that reflect specific alterations or abnormalities in brain function, and comprise several distinct categories. Some of the more common psychiatric disorders include the psychotic illnesses, which are associated with gross disruptions of normal cognitive functioning such as hallucinations, thought disorders and delusions; affective disorders, which are characterized by marked extremes of mood states such as severe depressed mood or mania and/or disruptive oscillations between different mood states; anxiety disorders, characterized by hypervigilance, arousal or fear out of proportion to external stimuli, as well as disorders of impulse control such as ADHD, substance use disorders and eating disorders.

It is important to recognize that even within a given diagnostic category, psychiatric disorders generally are classified on the basis of observed signs

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

and symptoms – these are syndromal diagnoses. The Diagnostic and Statistical Manual for Mental Disorders, 4th edition Text Revision (DSM-IV-TR)<sup>1</sup> states:

*[In DSM-IV] there is no assumption that all individuals having the same mental disorder are alike in all important ways. The clinician using DSM-IV should therefore consider that individuals sharing a diagnosis are likely to be heterogeneous... (p. xxxi).*

This heterogeneity means that two individuals with, for example, schizophrenia can present with quite different sets of signs and symptoms: one patient may experience paranoid delusions and auditory hallucinations, while another may present with disorganized thinking and loose associations. To complicate matters further, this heterogeneity is not confined to disease phenotype – it may also be that the pathogenesis and pathophysiology of a given psychiatric disorder vary among different individuals who share that diagnosis. From the perspective of drug discovery and drug development this is of particular importance, as it suggests that different patients with the same diagnosis may have quite different responses to a particular intervention.

Our current understanding of the etiology of most psychiatric disorders is imperfect. Characteristic alterations in certain laboratory or other biological measures have been shown in some disorders, but few psychiatric disorders have been associated with broadly reproducible pathophysiological findings that suggest a clear link to disease pathogenesis and etiology. With respect to the genetic bases of disease, many psychiatric disorders have been shown to have high heritability, and studies have suggested that some specific gene polymorphisms appear to be associated with increased risk for particular disorders. However, even for the genes with the strongest evidence of association to particular illnesses, the contribution to the overall observed phenotype attributable is likely to be small, suggesting that most psychiatric illness are the product of a complex interaction of genetic and environmental factors. Finally, most psychiatric disorders appear to have important developmental components in which experience and gene-environment interactions act together in disease pathogenesis.

## 1.2 Diagnostic Considerations

As noted above, and in contrast to other fields of medicine where diagnosis is based on pathophysiology or etiology, psychiatric diagnoses or diseases are predominantly syndromes. While the psychiatric research community continues to work toward defining specific mental illnesses based on pathophysiology and etiology, this goal has only been achieved for a limited number of disorders, such as many of the dementias (*e.g.*, Alzheimer's, multi-infarct or those due to other general medical conditions), delirium and substance-induced syndromes.

The process of diagnosis in psychiatry has been formalized through the development of structured classification systems. The most widely used classification systems are those based on the DSM-IV-TR<sup>1</sup> and the International Classification of Diseases (ICD-10) Classification of Mental and Behavioural Disorders,<sup>2</sup> both of which are currently under revision. These systems enable a

consistent and comprehensive approach for diagnosing psychiatric disorders. Within each system, the psychiatric diagnoses are categorized based on the most salient features, with further summary of each diagnosis including specification of the symptoms required to make a given diagnosis. Using these frameworks for psychiatric diagnosis serves several functions, notably reducing the complexity of these clinical phenomena, facilitating communication between clinicians and researchers, assisting in prediction of outcome and determination of appropriate treatment alternatives. Table 1.1 provides a listing of the various categories of psychiatric disorders as described in DSM-IV-TR.

**Table 1.1** DSM-IV-TR Classification.<sup>1</sup>

---

**Disorders usually first diagnosed in infancy, childhood or adolescence**

Mental retardation

Learning disorders

Motor skill disorder

Communication disorders

Pervasive Developmental Disorder

Attention-Deficit and Disruptive Behaviour Disorders

Feeding and eating disorders of infancy or early childhood

Tic disorders

Elimination disorders

Other disorders of infancy, childhood, or adolescence

**Delirium, dementia, amnesic and other cognitive disorders**

**Mental disorders due to a general medical condition not elsewhere classified**

**Substance-related disorders**

**Schizophrenia and other psychotic disorders**

**Mood disorders**

Depressive disorders

Bipolar disorders

**Anxiety disorders**

**Somatoform disorders**

**Factitious disorders**

**Dissociative disorders**

**Sexual and gender identify disorders**

Sexual dysfunctions

Gender identity disorders

**Eating disorders**

**Sleep disorders**

Primary sleep disorders – dyssomnias, parasomnias

Sleep disorders related to another mental disorder

Other sleep disorders

**Impulse-control disorders not elsewhere classified**

**Adjustment disorders**

**Personality disorders**

**Other disorders that may be a focus of clinical attention**

---

## 1.3 Treatment Options

At the present time, pharmacologically based approaches are considered the foundation of treatment for most psychiatric disorders. Developments in the field of psychopharmacology since the 1950s have provided considerable advances for the treatment of psychiatric illness. These developments have also served as tools for expanding our understanding of neurochemistry and for developing new disease classifications based on responses to specific drugs manipulating specific neurochemical targets in brain. Pharmacologic treatment considerations for some of the more common psychiatric disorders are as follows.

### 1.3.1 Schizophrenia

Antipsychotics have been the mainstay of treatment for schizophrenia since the development of chlorpromazine in the 1950s and the recognition of the role of dopamine in this chronic and debilitating disorder. Working primarily through dopamine receptor blockade, the first-generation antipsychotics represented an important advance in the treatment of schizophrenia, improving the positive symptoms which are hallmarks of the disorder (*i.e.* delusions, disordered thought and speech and perceptual disturbances), with benefits observed within one to two weeks. However, these drugs have little impact on the negative symptoms (*i.e.* blunted affect, alogia, anhedonia, asociality and avolition) and cognitive dysfunction, which are also characteristic of the disorder. The newer, “atypical” antipsychotics also demonstrate serotonergic activity, with less dopaminergic activity and, arguably, improved tolerability. Nonetheless, all antipsychotics have significant and unpleasant sideeffects which can limit their utility, including the following: extra-pyramidal symptoms and risk of tardive dyskinesia; weight gain, diabetes, dyslipidemia and risk of metabolic syndrome; galactorrhea and sexual dysfunction; haematologic effects (*e.g.*, agranulocytosis); and neuroleptic malignant syndrome. Clearly, alternative treatments with better tolerability and a broader spectrum of activity, improving both positive and negative symptoms as well as cognition, are needed. In recent years, considerable research interest has focused on the development of drugs targeting glutamatergic pathways; however, no successful development programs have emerged to date.

### 1.3.2 Major Depression

Approved pharmacologic treatments for depression act primarily through increasing synaptic availability of monoamines and/or direct interaction with monoaminergic receptors. In comparison to older generation tricyclic antidepressants (TCAs) and monoamine oxidase (MAO) inhibitors, the selective serotonin reuptake and serotonin norepinephrine reuptake inhibitors (SSRI and SNRI, respectively) and other newer antidepressants have broader therapeutic indices, thereby allowing for marked expansion of psychiatric



treatment into primary care settings. The full benefit of treatment may take 6 to 12 weeks, with initial improvement generally reported within 2 weeks. While awaiting the antidepressant response, short-term anxiolytic treatment may be co-administered. For patients who fail to respond to an optimal dose of an antidepressant, another class of antidepressant may be tried or the antidepressant can be augmented with another drug. As with antipsychotics, antidepressants are also associated with a number of treatment-limiting side-effects including sexual dysfunction, weight gain, insomnia, sedation, other psychiatric and neurologic symptoms, dietary restrictions (MAO inhibitors), lethality in overdose (TCAs and MAO inhibitors), increased risk of suicidal ideation and behaviour and discontinuation symptoms with abrupt discontinuation. Preclinical and clinical observations over the last several decades have implicated other neurochemical systems as therapeutic targets for depression, with potential roles for the following: hippocampal neurogenesis (neurotrophins); hypothalamic-pituitary axis dysfunction (CRF antagonists); immunologic dysfunction, with activation of pro-inflammatory cytokines; circadian dysfunction; and the role of oestrogen, given the increased prevalence of depression in women and alterations in mood that are observed during the reproductive years.

### 1.3.3 Anxiety Disorders

Pharmacologic treatment of anxiety primarily targets reducing the increased arousal and fear associated with the various anxiety disorders. Available pharmacologic agents that have demonstrated efficacy for anxiety disorders work primarily through increasing GABAergic tone and/or modulating serotonergic and noradrenergic transmission. Benzodiazepines have rapid onset of action, an important attribute for an anxiolytic, with variable duration of effect based on a given drug's half-life and metabolic profile (*e.g.*, presence of active metabolites), and many of these drugs need to be taken several times a day. Benzodiazepines can be associated with the development of tolerance, which, in turn, can lead to the need for dose escalation. When administered chronically, benzodiazepines are associated with withdrawal phenomena upon abrupt discontinuation and, therefore, tapering over time may be required for patients who wish to discontinue these medications. Other untoward effects include residual sedation, cognitive impairment, increased risk of falls in the elderly and respiratory depression and potential lethality in overdose. With the development of the TCA and SSRI/SNRI antidepressants, clinicians noted that patients also reported improvement in symptoms of anxiety. Efficacy of several antidepressants has subsequently been demonstrated for treating a variety of anxiety disorders, including panic disorder, social anxiety disorder, obsessive-compulsive disorder, post-traumatic stress disorder and generalized anxiety disorder. Unlike the benzodiazepines, however, SSRI/SNRIs can be anxiogenic upon initiation and onset of an anxiolytic effect may take several weeks. The other side-effects for SSRI and SNRI antidepressants described above are frequently even less well tolerated in patients with anxiety disorders. For more

refractory cases, atypical antipsychotics or MAO inhibitors may be indicated. The future of drug development for the treatment of anxiety disorders is challenged by the absence of well-defined, novel targets.

### 1.3.4 Insomnia

Insomnia may be viewed as a primary disorder of sleep or, alternatively, as a condition secondary to another underlying illness, such as sleep apnoea, major depression or substance abuse or dependence, for example. Secondary insomnias are best treated by addressing the underlying condition. In contrast, pharmacological treatment of primary insomnia is currently dominated by benzodiazepines (BZD), non-BZD hypnotics acting at the benzodiazepine site, sedating antidepressants such as trazodone and sedating non-prescription medications which are readily available over the counter (*e.g.* sedating antihistamines). As noted above, benzodiazepines have a number of properties and side-effects that have limited their use. In addition, the non-BZD treatments also have issues with tolerability, including next-day cognitive impairment related to residual effects of treatment. Recognizing the potential role of melatonin in regulation of sleep/wake cycle, recent work with melatonin agonists has shown benefit in treating transient circadian disturbances (*e.g.*, jet lag), but appear less promising for insomnia. Recent identification of novel compounds targeting orexigenic pathways directly linked to circadian biology offers promise for future therapies that overcome the limitations and disadvantages of those affecting the GABA<sub>A</sub> receptor system.

### 1.3.5 Bipolar Disorder

Bipolar disorder is characterized by periodic alterations in mood states, cycling between extremes of mood elevation and arousal, often accompanied by psychosis and depression. Pharmacologic treatment targets stabilization of mood and reducing the likelihood of cycling between mood states. The most widely prescribed mood stabilizers are lithium and various anticonvulsants (*e.g.*, lamotrigine and sodium valproate). Atypical antipsychotics have also demonstrated efficacy in treating mania. Antidepressants may be used in this population in conjunction with mood stabilizers, but with caution, due to the risk of precipitating mania.

### 1.3.6 Other Psychiatric Disorders

The development of pharmacologic treatments for other common psychiatric disorders has been hampered by our limited understanding of the underlying neurobiology and pathophysiology of these conditions and, thereby, inability to identify neurochemical targets for drug development. For example:

- Attention-deficit hyperactivity disorder (ADHD) is the most common neurobehavioral disorder in childhood, often manifesting during the early

school years and adversely impacting academic performance and normal psychosocial development and may persist into adulthood. The underlying pathophysiology for ADHD is unknown, but currently approved pharmacologic treatments include stimulants and, more recently, the selective norepinephrine reuptake inhibitor atomoxetine. While limited data are available regarding long-term outcomes associated with atomoxetine, stimulants are controlled substances with potential for abuse and the safety of their use in the long term continues to be a topic of debate and investigation.

- Autism is a pervasive neurodevelopmental disorder manifesting within the first three years of life and affecting the brain's normal development of social and communication skills. Research suggests a complex etiology, with contributions from genetic, neurophysiologic and environmental influences. There are currently no medications approved for treating patients with autism. Thus, medications are prescribed primarily for the symptomatic treatment of disruptive behaviours.
- Addiction encompasses disorders of the brain and behaviour associated with use, usually excessive, of psychoactive substances. Substances associated with addiction include widely available products such as nicotine, caffeine and alcohol, as well as over-the-counter (*e.g.* ephedrine) and prescription medications (*e.g.*, BZD, opiates, stimulants) and illicit drugs (*e.g.* cannabis, cocaine, heroin, methamphetamine, hallucinogenics). Disorders associated with misuse of psychoactive compounds are classified in psychiatry as substance-related disorders. These range from acute intoxication and withdrawal to more chronic abuse and dependence and are associated with other psychiatric and medical co-morbidity and psychosocial impairment. While researchers work to understand the biologic and genetic underpinnings of addiction, including the role of neural pathways associated with reward, pharmacologic treatment remains largely symptomatic. Available treatments focus on the effects of substance withdrawal in the absence of medications addressing prevention or progression of disease, cravings and maintenance of abstinence.

While many patients with psychiatric illness benefit from available treatments, others may demonstrate only a partial response or may fail to respond altogether. Positive outcomes are further challenged by the high rates of psychiatric and other medical co-morbidities, which complicate proper diagnosis and treatment. Currently available treatments are each associated with tolerability issues, which limit their utility in many patients. Poor tolerability, along with impaired insight associated with many psychiatric disorders, in turn contribute to treatment non-adherence and adversely impact outcome. Thus, many unmet medical needs remain.

## 1.4 Unmet Medical Needs

Pharmacotherapy for psychiatric illnesses has advanced remarkably in the last 50 years. Effective treatments for schizophrenia, depression, bipolar disorder,

the anxiety disorders and other debilitating psychiatric conditions are now available. Nonetheless, critical needs remain for new treatments. A very brief and incomplete survey of these needs would include:

- For schizophrenia, atypical antipsychotics can provide many patients with good control of acute signs and symptoms with fewer side-effects compared with first-generation antipsychotics. However, for many patients the most efficacious drugs are poorly tolerated or are associated with unwanted consequences such as weight gain and metabolic changes. Further, the more chronic and devastating sequelae of the illness, particularly deficit or “negative” symptoms and cognitive impairment, are not well treated by currently available drugs.
- Today’s antidepressants are safer and better tolerated compared with the earlier drugs such as tricyclic reuptake inhibitors and monoamine oxidase inhibitors. However, only about one-third of patients with depression respond fully to today’s pharmacologic treatments, and a similar number have little or no response. Thus, new drugs that could, alone or in combination with available drugs, induce a more complete and lasting response in poor and partial responders are badly needed.
- Patients with bipolar disorder have a number of mood stabilizers available to them. However, for many of the drugs, such as lithium and atypical antipsychotics, tolerability is a significant problem, and many patients have more complex presentations with symptoms that are inadequately controlled with available drugs.

Most other psychiatric disorders have similar gaps in the current therapeutic armamentarium, or in some cases, such as autism, have very little if anything available as effective pharmacotherapy. In this context, there is a great opportunity as a great challenge for those working to discover and develop new drugs to treat psychiatric disorders. The variety of unmet needs supports a number of different approaches. Thus, drugs that maintain today’s efficacy but improve safety and tolerability can be important in some disorders. In others, drugs that can be added to existing treatments to increase response would be highly valued by patients and physicians. And novel treatments, working through new mechanisms that can provide new alternatives for patients and especially for those patients who respond poorly or not at all to the drugs available today, are also badly needed.

The opportunity for today’s drug discoverers and developers is also a challenge. There are many promising and interesting mechanisms that could potentially lead to new drugs. However, because of the complexity of psychiatric disorders and the limits of current understanding of pathogenesis and pathophysiology, many and perhaps most biologically plausible mechanisms have little rigorous validation. Most psychiatric disorders have few clear analogues in the animal world, so that models most commonly try to replicate aspects of disorders, or particular symptom clusters, and few well-validated, predictive animal models are available reliably to screen new mechanisms.

## **References**

1. American Psychiatric Association, Diagnostic and Statistical Manual for Mental Disorders, American Psychiatric Association, Washington DC, 4th edn, Text Revision, 2000.
2. The ICD-10 Classification of Mental and Behavioural Disorders, World Health Organization, Geneva, 1993.



# **Section 1**

## **Schizophrenia**





## CHAPTER 2

# *The Pathophysiology of Schizophrenia*

ARMIN SZEGEDI<sup>a</sup> AND MICHAEL EGAN<sup>\*b</sup>

<sup>a</sup> Merck & Co., Inc., Department of Clinical Neuroscience, MRL, Building 34 Room A5045, 1E Lincoln Ave, Rahway, NJ 07065, USA; <sup>b</sup> Merck & Co., Inc., Department of Clinical Neuroscience, MRL, 351 Sumneytown Pike, UG4C-06, North Wales, PA 19454, USA

\*Email: Michael\_Egan@merck.com

## 2.1 History of Clinical Concepts

Schizophrenia as currently conceptualized in the current version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) constitutes a broad and heterogeneous spectrum of clinical symptoms or syndromes, both from the clinical and most likely also from the neurobiological perspective. Over the last century, various prominent figures have influenced the conceptualization of the disorder, mostly from a clinical perspective, while more recent authors have also integrated recent neurobiological findings in an attempt to clarify the underlying pathophysiology of this disorder (or group of disorders).

At the beginning of the twentieth century, at a time when no generally accepted nosological subdivision of severe mental disorders existed, the German psychiatrist Emil Kraepelin distinguished the group of manic-depressive illnesses from that of chronic psychotic illnesses (which he termed “dementia praecox”, thus emphasizing the early onset and a progressive deterioration resulting in early dementia) based on the longitudinal course of

the disorder. He divided the disorder into three subtypes (hebephrenic, catatonic and paranoid). Eugen Bleuler, a Swiss psychiatrist, championed the term “schizophrenia” in patients in whom he observed striking dissociation of cognitive and affective symptoms. He categorized the broad clinical spectrum into primary or “fundamental” symptoms (labelled with the “four As”: ambivalence, associative loosening, affective disturbance and autism), which he deemed to be essential for the diagnosis, while symptoms such as delusions and hallucinations were considered secondary and therefore not pathognomonic. In contrast, Kurt Schneider conceptualized the disorder with the emphasis on first-rank symptoms (pathognomonic, including specific hallucinatory experiences such as hearing one or more voices commenting on patient’s actions and thoughts, but also delusion of broadcasting of thoughts and feelings of external forces interfering with thoughts, behaviour or bodily functions). According to Kurt Schneider, the symptom domains deemed essential by Bleuler were considered second-rank symptoms, thus placing a fundamentally different emphasis within the spectrum of observable psychopathological symptoms.

The tension between these concepts could not be resolved on the level of descriptive/nosological psychopathology (which also included other great clinicians such as Karl Leonhard, who proposed a different systematic approach to psychoses). Starting in the 1970s, the concept of operationalized diagnoses became popular, primarily aiming at improved diagnoses using a clearly defined set of criteria for the establishment of a clinical diagnosis. Feighner’s Research Diagnostic Criteria were followed by the broader approach implemented in the DSM manual starting with the third edition (DSM-III) in 1980, which in essence still is the underlying principle of today’s and the future versions of DSM-IV and DSM-V. The operationalized criteria implemented elements of both the Bleulerian and the Schneiderian construct of schizophrenia. At the beginning of the 1980s, Tim Crow published his theory of at least two distinct subtypes of schizophrenia, with Type I being characterized by acute onset with predominantly positive symptoms, good response to antipsychotics, good prognosis and a postulated dopaminergic hyperfunction, whereas Type II schizophrenia is characterized by a chronic course, poor prognosis, cognitive impairment, inadequate response to antipsychotics and more structural-morphological abnormalities.<sup>1</sup> At about the same time, Nancy Andreasen conceptualized that the categories of positive and negative symptoms exist along a continuum of clinical and biological manifestations and represent opposite endpoints of a continuum, along which patients can be mapped. Patients with a preponderance of negative symptoms were described as tending to show ventricular enlargement and more cognitive impairment, while patients with a preponderance of positive symptoms tended to have normal ventricular volumes and less cognitive impairment.<sup>2</sup>

Subsequent research has widely supported the notion that most patients diagnosed with schizophrenia experience symptoms in the areas of *positive, negative, cognitive* and/or *affective symptoms*, and it is still a matter of further research to elucidate the relationship between symptoms on these dimensions in more detail. The concept of schizophrenia has continued to mandate that patients exhibit these

symptoms while they show no disturbances of orientation (“clear consciousness”), that the symptoms cannot be explained by a general medical condition or substance abuse and that a defined set of symptoms persists over a prolonged period of time (the criterion in DSM-IV-TR is at least six months).

*Positive* symptoms generally comprise hallucinations, delusions and disturbances of coordinated thinking and behaviour. These so-called psychotic symptoms are not exclusive to schizophrenia, but can occur in other disorders such as bipolar disorder or psychotic depression as well.

*Negative* symptoms represent deficits of volition, drive and motivation and largely resemble Bleuler’s “four As”. There have been a number of attempts to classify negative symptoms, *e.g.* the distinction between primary (deficit) symptoms as essential for the diagnosis of schizophrenia, and secondary negative symptoms, which can occur in other disorders or in association with positive symptoms and their treatment.<sup>3</sup>

In recent years, *cognitive impairment* associated with schizophrenia has generated substantial research activities. It is now well documented that schizophrenia is associated with primary cognitive deficits. Also, non-affected first-degree relatives of schizophrenic patients have a higher degree of impairment than normal controls, though the severity of these deficits is often more attenuated than in overtly ill patients. The National Institute of Mental Health’s Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative has defined the cognitive domains in which schizophrenia often leads to impairment, and has developed a cognitive test battery to measure the degree of impairment. The domains of the MATRICS battery include working memory, executive function, attention/vigilance, processing speed, verbal learning, visuospatial learning and social cognition.<sup>4</sup>

Both negative symptoms and cognitive impairment are more and more recognized as the main drivers of functional disability in schizophrenia.

The dimension of *affective disturbances* has recently gained increased interest. Van Os and Kapur describe symptoms of mania and depression as two out of five axes, along which patients with schizophrenia develop relevant symptoms that warrant clinical attention.<sup>5</sup>

The nosological or categorical approach to characterize schizophrenia as a disorder with clear boundaries to other affective or non-affective psychotic disorders has not been convincing as a tool to predict individual course, treatment response and ultimate treatment outcome. It has also not generated universally accepted subtypes of schizophrenia. A growing body of evidence shows a substantial overlap of various features (genetic risks, spectrum of presented symptoms, even longitudinal course) between individuals diagnosed with schizophrenia or individuals diagnosed *e.g.* with bipolar disorder, schizoaffective disorder or psychotic depression.

## 2.2 Epidemiology, Longitudinal Course and Outcome

Schizophrenia is a disorder with overall remarkably similar lifetime prevalence rates all over the world. A large number of studies have found a lifetime

prevalence of approximately 0.5–1% in various countries and geographical regions (not surprisingly, the prevalence rates appear to depend somewhat on the narrowness of the diagnostic definition). It is generally described as a disorder with the onset of the first manifest psychotic episode in late adolescence or early adulthood. In the prospective Mannheim study, Haefner reported that the age distribution at the earliest sign of mental disorder showed an early and steep increase until the age of 25 in males, and a somewhat delayed and smaller first peak in females, with a second peak in the age range of 45–79. Schizophrenia began with negative symptoms in 70% of cases, appearing two to six years before admission, and all positive symptoms appearing up to two years before.<sup>6</sup> Prior to the occurrence of the first psychotic episode, a number of prospective studies have reported the presence of non-specific symptoms that seem to precede the first psychotic exacerbation in many cases for several years and are conceptualized as a prodromal risk syndrome in individuals with high risk to develop psychosis. During this period, patients may experience non-specific symptoms such as loss of energy, a decline of their functional level, affective and behavioural symptoms, moderate but significant neuropsychological difficulties that pose in many instances substantial distress on the affected individuals and their social environment and may trigger them to seek professional help.<sup>7,8</sup> Evidence suggests that prospective ascertainment of individuals at risk for psychosis is feasible, with a level of predictive accuracy comparable to that in other areas of preventive medicine. The syndrome is currently considered for inclusion in the DSM-V manual under the diagnostic label of “attenuated psychosis symptoms syndrome” as a psychosis risk syndrome.<sup>9</sup>

Schizophrenia is in many cases a chronic and recurring disorder. Individual courses may vary substantially, as the psychopathological symptoms can vary both between the individuals, and also within a subject depending on what stage of the disorder the individual is going through. After an often prolonged prodromal phase of attenuated symptoms, the first acute exacerbation often features an abrupt onset of positive symptoms with prominent delusions, hallucinations and changes in the thinking and behaviour of the patient, which often appear disorganized, bizarre or incoherent. If treated successfully, positive symptoms can often resolve to a remarkable degree or even remit completely. Yet in many cases, symptoms of impaired cognitive skills, negative symptoms and/or continued impairment of functionality can be observed after the acute episode subsides. While approximately 20–25% of patients with a first episode may never experience a subsequent episode, a majority of patients will. Recurrent episodes may lead to complete or almost complete resolution of symptoms in the interval between them, but the general tendency appears to be that an increasing number of episodes increases the probability of incomplete symptom resolution and to the presence of residual symptoms. In approximately one-third of the patients, a chronically deteriorating longitudinal course is observed, with a decrease of cognitive abilities and an increased level of negative symptoms, which often translate into a loss of functional capabilities such as the ability to be professionally productive or to be socially well integrated.

According to systematic analyses led by the World Health Organization, schizophrenia is one of the leading causes of years lived with disability, ranking among the top seven causes across all age groups and within the top three in the age group of 15–44 years old.<sup>10</sup> It is associated with an enormous burden for the individuals, their social environment and the societies in which patients live.

Schizophrenia is associated with a substantially increased risk for premature death. Patients with schizophrenia die approximately 15 years earlier than non-affected people.<sup>11</sup> While suicide is an important risk factor for premature death, in most cases patients suffer from concomitant medical conditions that lead to their reduced life expectancy, such as consequences of heavy smoking, obesity and metabolic disorders, which are often linked to a lifestyle of unhealthy nutrition or lack of exercise facilitated by the clinical symptoms of schizophrenia.

## **2.3 Etiological Concepts for Schizophrenia**

The etiology of schizophrenia is still poorly understood. The current syndromal concepts of schizophrenia are largely based on a collection of signs and symptoms in conjunction with criteria that relate to a minimally required duration of symptoms, and the absence of a medical condition or substance use as more likely explanations of the observed clinical phenomena. The heterogeneity of the symptoms and the fact that they do not occur exclusively during schizophrenia complicate their straightforward explanation on a neurobiological level even further.

Current attempts to clarify how schizophrenia develops in an affected individual usually assume a complex interaction between environmental factor and genetic predisposition. While there is strong evidence that genetic factors have a role, the complexity of involved mechanisms are not well understood, but for single genetic markers an important modulatory role in neurophysiological functions has been reported, which may pave the way for defining more homogenous neurobiological endophenotype in the future.

In terms of environmental factors an extensive list of suspected contributing factors has been proposed and examined, some of which have been dismissed over time (“the schizophrenogenic mother”), while others are still debated in their exact role (*e.g.* obstetric complications, season of birth effects, slow virus infections, urban birth, migration, cannabis consumption).

The steadily aggregating amount of data from various disciplines, particularly neuroanatomical and neuroimaging<sup>12</sup> data, has generated in recent years an increasing interest in the concept of schizophrenia as a neurodevelopmental disorder, initially proposed by Weinberger.<sup>13</sup> As currently conceptualized, the model implies a trajectory of illness with different stages.<sup>14</sup> Stage 1 is characterized by the presence of genetic vulnerability and exposure to environmental risk factors, yet the symptoms may be absent or mild, and no intervention has been identified so far. Stage 2 would resemble a clinical situation similar to the attenuated psychosis symptoms syndrome, or prodromal syndrome, with cognitive and behavioural deficiencies, but without

symptoms meeting current criteria for the diagnosis of schizophrenia. Stage 3 would resemble the acute stage of schizophrenia, including relapsing-remitting courses in the longitudinal course, with the associated incapacitating impact on functioning levels. Stage 4 resembles the chronic residual state, with chronic disability, psychosocial sequelae such as loss of function, unemployment or homelessness.

Currently established therapies mainly focus on stages 3 and 4, with an increasing amount of data emerging for stage 2.

## 2.4 Treatment of Schizophrenia

The serendipitous discovery of antipsychotics in the mid 1950s has fundamentally changed the fate of people affected by schizophrenia. Prior to the introduction of compounds like chlorpromazine and later haloperidol, symptoms of psychosis were largely not responsive to available treatments, and many patients were subjected to harsh interventions that in retrospect appear very drastic, yet of marginal therapeutic value (*e.g.* lobotomy, insulin shock therapy). Antipsychotic drugs lead in many cases to a marked reduction of psychotic or positive symptoms. Their efficacy, however, seemed to go hand in hand with the induction of extra-pyramidal movement disorders, such as Parkinson-like rigidity, dystonia and the inability to sit still (akathisia), which were soon discovered to be due to extensive dopamine receptor blockade, the effect that seemed also to mediate efficacy. For a while efficacy and extra-pyramidal symptoms were considered necessarily linked to each other, until clozapine was discovered as the first drug to behave “atypically” in this sense, as it generally does not induce EPS. Since the mid 1990s a number of pharmacologically and structurally distinct compounds have been developed, which share as a common feature the reduced rate of induced EPS, and are therefore often termed “atypical antipsychotics” (despite its appeal at first sight this category is more problematic and debated in scientific literature than is often recognized<sup>15</sup>) or “second-generation antipsychotics”. They include compounds like olanzapine, risperidone, quetiapine, ziprasidone, aripiprazole and asenapine. While these compounds do not show marked differences in terms of clinical efficacy on a group level, they markedly differ in their side-effect profiles,<sup>16</sup> which appear more directly related to their pharmacological profile. It is important to note that the common assumption that antipsychotic drugs having similar overall efficacy also means that each antipsychotic drug will have about the same clinical effect in each patient is plain wrong. For reasons that are not well understood, individuals in clinical practice may react very differently to different compounds, in terms of both tolerability and also efficacy. It is one of the major challenges in practice for a clinician to find a drug treatment for an individual patient that provides sufficient symptom control as well as acceptable short- and long-term tolerability and safety.

It is currently well established that antipsychotics are effective in reducing primarily positive symptoms, and their use can stabilize acutely ill patients and prevent acute exacerbation in a significantly higher number of patients than



without continuing medication treatment. Adherence to antipsychotic medications is a very important factor for increasing the likelihood of success for rehabilitation efforts of patients after successful symptom control in the acute phase. The use of depot formulations may be particularly helpful in assuring therapeutic drug exposure levels, as schizophrenia itself is a disorder that has an inherent risk of treatment non-compliance. However, currently available treatments have so far largely failed to demonstrate tangible clinical efficacy in the domains of negative symptoms or cognitive impairment, which drive a substantial part of the loss of functionality in patients with a chronic course of the disorder. While some degree of alleviation may be achieved in some patients, especially with prolonged treatment over several months or even years, controlled data have largely failed to show superiority of these drugs over control treatments. This is clearly an unmet medical need for future drug development, and also a very challenging one to tackle.

More recent drug development efforts aiming to treat symptoms domains in schizophrenia have focused on targets affecting glutamatergic neurotransmission, *e.g.* glycine uptake inhibitors or mGluR2/3 agonists. They are discussed in Section 2.5.2.

## 2.5 Neurochemical Abnormalities

The earliest and, perhaps, still most compelling theories about the biological basis of schizophrenia propose that neurochemical abnormalities play a key role. Of these, the dopamine hypothesis has dominated the conceptual landscape. Initial formulations of the dopamine hypothesis posited simply that schizophrenia was due to increased striatal dopamine neurotransmission. Since then, more sophisticated revisions have proposed, for example, that cortical dysfunction, including reduced cortical dopamine, lead to downstream elevations in subcortical dopamine. Abnormalities in a second neurotransmitter system, the glutamate system, have also been implicated, albeit by relatively indirect evidence, such as the similarity between schizophrenia and the psychosis induced by NMDA antagonists. Two other neurotransmitters, GABA and serotonin, have also received substantial attention and may play a role. Beyond these four, many other neurotransmitters, neuromodulators and neurochemicals have been suggested to play a role, but supportive studies are less extensive or conflicting.

A recurring challenge in the studies investigating neurochemical theories, including *post mortem* and imaging studies, is that a number of confounding factors often cloud the interpretation of their results. These include patient use of antipsychotic and other medications, their high rates of nicotine and substance abuse and their relatively poor health status (*e.g.* increased rates of head injury, diabetes, *etc.*) compared to control subjects. These issues have required additional studies of animals treated with antipsychotics, patients withdrawn from antipsychotic medications, and unmedicated “first break” subjects (prior to treatment with antipsychotics), as well as studies of unaffected first-degree

relatives, which can implicate genetic factors. More recently, genetic approaches have also been used, which are immune to these particular confounds. Overall, a compelling body of literature has emerged to support the notion that alterations in dopamine, glutamate, GABA and perhaps serotonin play a role in the expression of schizophrenia. Whether these abnormalities are primary or downstream consequences of some other process remains uncertain.

## 2.5.1 Dopamine

The dopamine hypothesis initially emerged as the result of several key findings in animal models and early clinical observations in patients. First, Carlsson and Lundquist showed in the early 1960s that drugs with antipsychotic effects increased the metabolism of dopamine in rodents, suggesting their effects are mediated through dopamine. Second, clinical reports suggested that dopamine agonists, such as amphetamine, when given chronically and at high doses can induce a paranoid psychosis in healthy subjects, similar in some respects to the psychotic symptoms seen in schizophrenia. Further studies showed that antipsychotics all produced Parkinsonian-like side-effects in patients, indicating that they reduced striatal dopamine neurotransmission. A seminal discovery came in the 1970s with the demonstration that the affinity for the D<sub>2</sub> receptor predicted the clinical potency of a wide chemical array of antipsychotics.<sup>17,18</sup> This finding confirmed unequivocally that the D<sub>2</sub> receptor was involved in the expression of acute psychotic symptoms. Since then, D<sub>2</sub> has become the primary target for drug discovery efforts.

Subsequent to these early findings, an avalanche of research has provided remarkable insights into the nature of both pre- and post-synaptic dopamine dysfunction in patients as well as the regulation of dopamine neurotransmission in studies using rodents and primates. Initially, *post mortem* studies focused on the D<sub>2</sub> receptor and found increased receptor density in the striatum, but rodent studies suggested this may be due to prior neuroleptic treatment. PET studies using D<sub>2</sub> ligands also found increased D<sub>2</sub> density in chronically ill patients, but, again, neuroleptic effects could not be excluded. However, follow-up PET studies of unmedicated patients, including never-treated patients, found similar increases in D<sub>2</sub> density. Overall, as reported by several recent meta-analyses, it appears most likely that patients have a 10–20% elevation in D<sub>2</sub> density, which is not the result of prior neuroleptic treatment. Genetic data support this notion as some D<sub>2</sub> alleles may slightly increase risk for schizophrenia. In contrast to D<sub>2</sub>, studies of other dopamine-related proteins, including receptors (e.g. D<sub>1</sub>, D<sub>3</sub>, D<sub>4</sub>), transporters (e.g. the dopamine transporter, DAT) and enzymes (TH, MAO) have generally been negative. One exception is COMT, which may be particularly important for modulating prefrontal dopamine tone (see below).

In addition to increased post-synaptic D<sub>2</sub> density, schizophrenia may also involve increased pre-synaptic dopamine storage and release. A major advance came from second-generation PET imaging studies, which used l-dopa to label pre-synaptic terminals. These studies found increased storage and pre-synaptic



synthesis rates in schizophrenia, strongly implicating a pre-synaptic deficit. Next, seminal studies by Laruelle and Abi-Dargham found consistent evidence for increased pre-synaptic dopamine release. Their studies used drug challenges with amphetamine combined with neuroimaging using  $D_2$  ligands to assess the  $D_2$  off rates and, by implication, the synaptic release of striatal dopamine. Patients showed marked increases in the displacement of  $D_2$  ligands, implying increased pre-synaptic vesicular storage of dopamine. These findings have been replicated by other groups and have also been reported in subjects with schizotypal personality disorder as well as unaffected first-degree relatives of patients with schizophrenia.

In parallel to these clinical efforts, studies using animal models have explored the regulation of striatal dopamine release and midbrain dopamine neuronal activity in more detail. Two types of dopamine firing have been described: tonic, which is dependent on low, predictable neuronal firing, and phasic, which is dependent on an intermittent burst pattern of firing. These firing patterns convey different types of information. Tonic release may regulate behavioural flexibility, while phasic release may be more important for the experience of reward and may underlie associative learning.<sup>19</sup> The two types of dopamine release are regulated by different mechanisms and inputs from different regions. Phasic release, for example, in animal models, is dependent on NMDA-mediated glutamate neurotransmission from the pedunculopontine tegmentum. A provocative and influential variant of the dopamine hypothesis proposed by Grace suggested that in schizophrenia reduced tonic release (perhaps due to prefrontal dysfunction) is accompanied by increased phasic release.<sup>19</sup> Although there is little clinical evidence to support this notion, it has been heuristically useful.

A critical brain region regulating midbrain dopamine neurons is the prefrontal cortex (PFC). Some, but not all, studies in rodents, primates and humans have found that impaired PFC function can produce altered striatal dopamine, with evidence for both increased presynaptic stores as well as increased presynaptic release. These studies have provided a lynchpin for revisions to the dopamine hypothesis, namely that abnormalities in striatal dopamine are secondary to PFC dysfunction. Evidence for PFC impairment in schizophrenia is substantial. Impaired prefrontal cognition (*i.e.* working memory/executive function) is a common if not ubiquitous feature of the illness. *Post mortem*, neuroimaging and electrophysiological studies (see below) have found reduced volume, reduced neuropil, impaired PFC efficiency and reduced signal to noise, which likely underlie the impairments in cognition. Provocatively, some studies suggest that dopamine innervation and/or  $D_1$  receptor density are reduced in the PFC, although these studies have not been well replicated. These findings have fuelled speculation and revisions to the original dopamine hypothesis with the notion that a hypo-dopaminergic PFC leads to a hyper-dopaminergic striatum. Support for abnormal  $D_1$  PFC signalling comes from seminal studies of non-human primates by Goldman-Rakic<sup>20</sup> showing that  $D_1$  receptors tune cortical neurons and that reduced  $D_1$  signalling results in reduced signal-to-noise ratio, consistent with clinical

findings in patients using fMRI and EEG (see below). Support for reduced PFC dopamine leading to impaired cognition and increased subcortical dopamine also comes from a series of convergent genetic studies in humans using the val/met polymorphism of the COMT gene, which appears to exert a substantial effect on synaptic dopamine in the PFC and downstream but opposite effects in the striatum.<sup>21–23</sup>

A third region implicated in dopamine dysregulation is the mesial temporal lobe, including the hippocampus and related structures. These areas receive dopamine innervation from the midbrain mesolimbic system. Preclinical studies have shown that hippocampal projections to the striatum exert a strong modulatory influence on dopamine neuronal firing. Similar to the PFC, medial temporal lobe abnormalities have been reported in schizophrenia using a variety of experimental techniques. Furthermore, rodent and primate studies have shown that hippocampal lesions can produce downstream impairments in PFC and striatal dopamine.<sup>24</sup> Taken together, the triad of mesial temporal lobe, PFC and striatum represents the most compelling set of circuits likely to be related to dopamine abnormalities and pathophysiology.

In summary, dopamine dysfunction in schizophrenia may be, in part, primary, and in part the downstream consequence of cortical dysfunction in several brain regions. Impaired prefrontal function, perhaps due to reduced D<sub>1</sub> signalling, may cause reduced tonic release in subcortical structures. This may increase the gain for phasic release and, acting through increased striatal D<sub>2</sub> receptor density, may conspire to produce inappropriate learning and misattribution of the salience of environmental stimuli. Reduced PFC dopamine could be due to genetics or to developmental abnormalities, including those in other brain regions, such as the hippocampus. Conversely, primary alterations to striatal dopamine could produce PFC abnormalities. While it seems highly likely that the dysregulated dopamine system plays a key role in schizophrenia, the primary causes of this dysfunction remain largely unknown. More importantly, therapeutic interventions are still needed to correct the underlying dopamine abnormalities in a more physiologically relevant way so as to normalize dopaminergic regulation of motivation, affect and cognition.

### 2.5.2 Glutamate

A second major neurotransmitter system implicated in schizophrenia is the glutamate system. Support for the glutamate involvement was initially based on similarities between neuropsychiatric symptoms of schizophrenia and acute intoxication with PCP, a non-competitive NMDA antagonist. Follow-up studies showed that all compounds that inhibit NMDA function, including those that bind to the glutamate and the glycine modulatory site, induce psychosis.<sup>25,26</sup> Efforts to elucidate the nature of glutamatergic abnormalities have followed several paths. *Post mortem* studies have evaluated glutamate receptor number and gene expression but the results have been mixed and no clear, replicable findings have emerged. *Post mortem* studies have also assessed glutamate neuronal number and synaptic density. These studies have suggested

that cell size and synaptic density may both be reduced, particularly in the PFC, but replications have been limited. *In vivo* imaging techniques have attempted to assess glutamate function in patients. These also have not yet produced clear-cut findings. Magnetic resonance spectroscopy, for example, has reported reductions in signals related to glutamate, but replication has been challenging. PET studies looking at receptor binding *in vivo* have only recently begun but hold promise for the future.

Preclinical studies have investigated the neurobiological effects of psychotogenic NMDA antagonists in an effort to further assess new treatment approaches as well as the precise pathophysiological mechanisms. For example, pharmacological studies have found that the deleterious behavioural effects of NMDA antagonists in animals could be blocked with mGluR2/3 agonists.<sup>27</sup> One possible mechanism for this effect is through reducing pre-synaptic glutamate release. NMDA antagonists have been reported to increase in glutamate release, suggesting that some of the psychotogenic effects may be due to over-activation of non-NMDA glutamate receptors, such as AMPA receptors. A key clinical trial of a Lilly mGluR2/3 agonist in schizophrenia found efficacy similar to that obtained with olanzapine.<sup>28</sup> Further replication could provide compelling evidence for glutamate's role in schizophrenia. Clinical trials have also been conducted using agonists at the NMDA receptor's glycine modulatory site, including glycine, D-serine and D-alanine. Small, pilot studies have suggested that these agents improve negative symptoms and cognition but, to date, no large, multi-site trial has replicated these findings, raising substantial doubts about the effectiveness of this approach. Similarly, inhibitors of the glycine uptake site (GlyT 1 inhibitors) have also been attempted but the results have been mixed.

Modulation of glutamate neurotransmission is complex and, in addition to NMDA, many facets of glutamatergic synapse have been interrogated. The glycine modulatory site, as mentioned above, has been a prime target as have processes related to glycine synthesis and degradation. Serine, a precursor to glycine, has been evaluated for its potential therapeutic effects, although results have not been compelling. Regulation of glutamate is pharmacologically rich with many potential drug targets. Another promising target is the mGluR5 receptor, but neurotoxic effects raise questions about this approach. Others include the mGluR3 receptor, which appears to modulate glutamate uptake sites, glutamate transporters, NAAG, NRG1 and ErbB4. Most data implicating these targets come from animal studies. Validation in human studies is very limited, although genetic and imaging approaches may be useful in this regard.<sup>29</sup> Furthermore, a major challenge with glutamate approaches is the well-known risk of neurotoxicity. Nevertheless, glutamate continues to hold allure in its promise of new therapeutic approaches to schizophrenia.

### 2.5.3 GABA

Abnormalities in GABA have been implicated for several decades, primarily by *post mortem* studies, with relatively little traction until recently. In general, GABA agonists and antagonists do not exert pronounced psychotogenic

clinical effects, compared to dopamine agonists and NMDA antagonists. Imaging studies using GABA ligands have unfortunately not been pursued. Regarding *post mortem* studies, a variety of alterations have been claimed, most of which have been difficult to replicate. For example, reduced density of cortical GABA interneurons, as well as developmental abnormalities of GABAergic neuronal migration, have been reported, but neither have been widely replicated. On the other hand, deficits in reelin have been more reliably observed in PFC. Reelin regulates development of cortical GABAergic neurons and deficits in reelin can reduce GABAergic synapse number. The reelin gene may also harbour alleles that increase risk for schizophrenia. By far the most highly replicated finding, and the finding that most strongly implicates GABA, is that of reduced PFC levels of mRNA and protein for GAD67, a key enzyme for GABA synthesis. These reductions may be due to abnormal methylation or to polymorphisms in the GAD1 gene. Further support for GABA dysfunction comes from an influential series of studies by Lewis and colleagues.<sup>30</sup> Using *post mortem* material, they have described a series of related abnormalities in GABAergic regulation of glutamate function. Specifically, in the PFC, parvalbumin-containing GABAergic basket cell terminals show reduced expression of the GABA uptake transporter, GAT1. On the post-synaptic side, up-regulation of the GABA alpha 2 receptor at the post-synaptic axon initial segment is observed. These findings *in toto* suggest reduced level of inhibition of cortical glutamatergic pyramidal neurons, which could account for abnormal pyramidal cell firing, the PFC “inefficiency” observed in fMRI studies (see below), and perhaps other downstream deleterious effects.

### 2.5.4 Serotonin

Serotonin was initially implicated by observations that hallucinogens, such as LSD, are serotonin agonists, particularly at the 5-HT<sub>2A</sub> receptor. *Post mortem* and genetic studies have evaluated serotonin receptors and related proteins (*e.g.* the serotonin transporter) in schizophrenia. These studies have also suggested a role for the 5-HT<sub>2A</sub> receptor. On the other hand, *in vivo* studies of patients using PET ligands have not found changes in 5-HT<sub>2A</sub> binding. Importantly, this receptor has also been implicated in the unique therapeutic effects of clozapine (see above).<sup>31</sup> Based on this notion, second-generation antipsychotics, developed in an effort to mimic clozapine’s superior efficacy, were designed as both 5-HT<sub>2A</sub> and D<sub>2</sub> antagonists. Furthermore, a pilot clinical trial suggested that a 5-HT<sub>2A</sub>/5-HT<sub>2C</sub> antagonist produced antipsychotic effects in acutely ill patients. Attempts to further capitalize on the potential therapeutic efficacy of 5-HT<sub>2A</sub> antagonists without D<sub>2</sub> effects, however, have not been successful.

## 2.6 Neuropathology

Decades of research in schizophrenia have not identified a consistent neuropathology but clues persist and offer hope that advances in methodology could

change this. It is clear from neuroimaging studies that brain volume is reduced and ventricles are enlarged in schizophrenia. The anatomical substrate that accounts for this reduction has been difficult to pinpoint. Perhaps the most commonly accepted finding is that there is no gliosis or other evidence of neurodegeneration. As a result, some have suggested that the neuropathological insult must occur *in utero*, during the second trimester, when gliosis does not occur following neuronal death. Recent studies have tended to focus on the hippocampus and PFC, regions implicated most often in neuroimaging studies. On a microscopic level, both areas look remarkably normal. In both regions, several reports have found evidence for altered cellular positioning or orientation, suggesting abnormal neuronal migration; however, further studies have clearly not observed these. More subtle abnormalities in connectivity or synaptic architecture have been suggested. Perhaps the most influential set of findings is a subtle reduction in neuronal density, neuronal size, dendritic complexity or synaptic density. In the PFC, relatively consistent findings are increased neuronal density and reduced synaptophysin, a marker of synapse number. The former finding has been interpreted as being due to reduced neuropil, presumably due to reduced density of dendrites and axon terminals. Unfortunately, this attractive hypothesis is supported by relatively few studies.

Several new methodologies are being applied to the study of *post mortem* material. While these are still in the early stages of development, they hold significant promise. First, improved unbiased stereological techniques are being developed to evaluate more subtle alterations and may provide future insights to changes to cerebral micro-architecture. Next, high-throughput “omic” methods evaluating expression of the entire genome, proteome, transcriptome and metabolome, as well as sequence variants of all expressed transcripts, are being used to develop a map of alterations in entire molecular networks within specific brain regions. While exciting, these new methods have not circumvented the traditional confounds (*e.g.* neuroleptics treatment, substance abuse) plaguing *post mortem* research, as mentioned previously. On the other hand, assessing the functional effect of allelic variants associated with illness on brain expression levels does not necessarily require the use of material from patients. Kleinman and colleagues have pioneered the study of genes that increase risk for schizophrenia and their impact on gene expression and downstream molecular mechanisms.<sup>32</sup> The recent publication of a brain expression atlas spanning the entire age range from *in utero* to old age is an important new tool for understanding changes in molecular networks and potentially validating new drug targets.<sup>33</sup>

## 2.7 Brain Imaging and Electrophysiological Abnormalities

A variety of brain imaging and electrophysiological techniques have been used to study the neurobiology of schizophrenia. Of these, structural brain imaging has yielded perhaps the most important findings. Beginning in the 1970s, CT

scans revealed that patients had substantial increases in lateral ventricular size as well as loss of brain volume. Subsequently, more refined MRI techniques were used to characterize more precisely the extent, location and timing of reduced brain volume. These findings had a substantial impact on the field. Psychological and psychoanalytic theories, already under assault from the dopamine-centric biochemical theories, were abandoned, and the view that schizophrenia was a biologically based brain disorder was unequivocally accepted. Well over 100 studies have documented the nature of the volumetric abnormalities. The location of the deficits appears to be multi-focal, or perhaps diffuse. Gray matter volume is reduced by roughly 3–4%. PFC and mesial temporal lobe volume is reduced by roughly 5%. Lateral and third ventricular volumes are increased by 30%. In contrast, some subcortical structures have increased volumes, which have been attributed to antipsychotic treatment. Some studies have also reported cortical asymmetries and abnormalities in cortical sulcal folds.

The issue of heterogeneity has been an important one regarding all biological findings in schizophrenia. Given the presumption that schizophrenia is a heterogeneous disorder, a natural question is do all patients have structural abnormalities or only a subgroup? A number of experimental approaches have been brought to bear on this question, perhaps more so for volumetric abnormalities than for any other biological finding. Although the data are not definitive, it appears that most patients are likely to have volumetric abnormalities compared to where they would be if not ill. Further attempts at correlating global or specific regional volumetric deficits with clinical symptomatology, however, have led to inconclusive results. Some studies suggest that positive symptoms are more closely related to temporal lobe deficits while PFC deficits are more related to negative symptoms.

Regarding the timing of the structural lesion, studies of at risk and first-break subjects have found that abnormalities are present from very early in the illness. This observation suggested that some neuropathological process preceded the clinical symptoms in schizophrenia and was instrumental in giving birth to the neurodevelopmental hypothesis (see below). Similar structural abnormalities have been observed, albeit to a lesser extent, in healthy family members, suggesting that they are not due to medications or deleterious environmental factors but may, in part, have genetic origins. Some but not all studies have reported progressive loss as the illness progresses. The extent of progressive volume loss is likely limited and substantially less than that seen in Alzheimer's disease.

Functional imaging studies have used FDG PET, which measures glucose utilization, or more commonly, fMRI, which looks at changes in blood oxygen, thought to be related to neural activity. Both techniques typically use cognitive tasks to activate and interrogate specific brain regions, compared to a baseline cognitive task, typically a simple task that matches the sensory and non-cognitive demands of the activation task. The most commonly studied region is the PFC, where working memory activation tasks are used to increase neuronal activity. Although abnormalities are clearly present, both increases and reductions in PFC activation have been reported, particularly the dorsolateral



PFC. The reasons for the discrepant findings are unclear, but may have to do with subtle differences in cognitive tasks and other experimental variables. One attractive attempt at synthesizing the results has proposed that inefficient neural computational activities lead initially to over-activation (“inefficiency”), followed by under-activation, or “hypoactivity”.<sup>34</sup> The implication is that PFC activation follows an inverted U-shaped curve, where activation increases up to a point, where function is optimal. When cognitive load is pushed beyond this optimal capacity, activation falls, on the down side of the curve. Remarkably, a similar inverted U-shaped curve has been observed in the seminal work of Goldman-Racik, who showed that in primates D-1 tone predicted one’s location on the inverted U-shaped curve.

Other regions have been examined using fMRI including subcortical regions, amygdala, cingulate, hippocampus and medial temporal lobe, as well as connectivity between these regions. Many studies have found abnormal activation in these regions, although not consistently, and confounding factors, as discussed above, have not been closely evaluated. Multi-variate approaches also show interesting patterns of abnormalities separating patients from controls.<sup>35</sup> Overall, these findings implicate a wide variety of cortical information-processing deficits, consistent with the wide range of cognitive deficits (see above). Perhaps more importantly, imaging has emerged as a useful tool to examine the impact of allelic variants in candidate genes on brain structure, function and related cognitive tasks.<sup>36</sup> Imaging genomics therefore may offer a powerful new approach to validating drug targets while providing translational biomarker tools for use in drug development.

Electrophysiological studies of schizophrenia have been undertaken for decades but began intensively in the early 1970s. A variety of subtle abnormalities have been reported in patients, and first-degree relatives. These include event-related potentials (ERPs) and sensory gating (*e.g.* modulating the magnitude of the startle response to an unexpected loud noise). Most ERP studies have used auditory stimuli to generate the ERP waveform. Primary outcome measures have included amplitude, latency and suppression of the waveform when two consecutive stimuli are presented (a variant of sensory gating). Some findings have not been well replicated or suffer from the many potential methodological confounds affecting other biological research in schizophrenia.

One of the first abnormalities reported was the reduction in the N100 ERP, a negative wave seen 100 ms after an auditory stimulus during an oddball paradigm. These commonly used paradigms elicit ERPs using an auditory stimulus presented 80% of the time followed by an “oddball” stimulus presented 20% of the time. The oddball is usually slightly different in pitch or tone compared to the more frequent stimulus. Patients with schizophrenia have a reduced amplitude in the N100 waveform to oddball stimuli. This N100 response is often referred to as the mismatch negativity (MMN) and may be in part genetic. Generators of this waveform appear to be in the temporal lobe and may be correlated with structural deficits there. MMN may be in part mediated by glutamate receptors. A second intensively studied waveform is the P300, a positive waveform seen 300 ms after an auditory stimulus. The P300 is also

reduced in the oddball paradigm in patients with schizophrenia. Pharmacological studies suggest P300 amplitude is mediated by acetylcholine. The biology of the P300 appears complex, and there are likely to be many cortical generators that produce the waveform. Family studies have reported a genetic basis. Thirdly, the P50 ERP has also been extensively studied. The amplitude of P50 is reduced in schizophrenia in response to the second of two auditory stimuli (clicking sounds) presented within 500 ms.<sup>37</sup> The P50 deficit is found in first-degree relatives, suggesting it is genetic, and is thought to be mediated by the alpha 7 nicotinic receptor, supporting this as a drug target. Several drug trials with alpha 7 agonists are ongoing.

## 2.8 Genetics

### 2.8.1 Heritability of Schizophrenia

One of the earliest biological observations about schizophrenia is that it runs in families. Numerous studies have clearly shown that the risk of schizophrenia is increased in relatives. The pattern of risk suggests it is not a classical Mendelian disorder. The concordance rate for monozygotic twins is about 50%, indicating that the penetrance of risk alleles is incomplete. Concordance for dizygotic twins is about 18%, while that for siblings and other first-degree relatives is about 10%. This suggests that dizygotic twins share an environmental factor that affects risk. These family data unequivocally demonstrate that risk factors are familial, but adoption studies were needed to directly implicate a genetic component. Although adoption studies are challenging, several have been completed and indicate that most if not all of the familial risk is heritable.

The pattern of family concordance rates can be used to infer the genetic architecture. A simple approach to estimating overall heritability is to use twice the difference in concordance rates between monozygotic (50%) and dizygotic (18%) twins, which is  $>60\%$ . Meta analyses of twin studies report heritability of  $>80\%$ .<sup>38</sup> This substantial genetic component of risk is not likely due to a single gene as with simple autosomal dominant and recessive disorders. Instead, the inheritance pattern suggests it is a complex oligogenic or polygenic disorder, perhaps involving genes by environmental interactions, or epistasis between two or more genes. Several environmental risk factors have been implicated, such as *in utero* infections, starvation, marijuana use or growing up in a city, which could interact with risk genes.

Two classic approaches for finding genes that impact risk for disease are linkage and association. Linkage, the classic approach, typically involves studying large families with many affected and unaffected members. Analysis of their DNA using genetic markers is used to assess which chromosomal regions are shared by the affected members. These regions may be very large, up to several megabases in size, and could potentially harbour dozens of genes. Once promising linkage results are obtained with a standard set of markers, detailed follow-up efforts are required to pinpoint the specific gene and mutation that cause disease, an effort that can be quite substantial and challenging.



In contrast to linkage, association studies typically compare patients and controls on the frequency of genetic polymorphisms, such as single nucleotide polymorphisms (SNPs). Association studies are easier to recruit than linkage studies and also have substantially more power (in theory) for finding genes. Initial studies using this approach focused on candidate genes implicated through studies of the biology of schizophrenia, such as  $D_2$  and  $5-HT_{2A}$  receptors, or other proteins related to dopamine (e.g. COMT), glutamate, GABA and serotonin. More recently, genome wide association studies (GWAS) have used chip-based technology to assay hundreds of thousands of SNPs covering the entire genome. While theoretically appealing, this approach requires a substantial statistical correction for the number of comparisons made.

Another approach employed to dissect the complex genetic architecture of schizophrenia is the study of intermediate phenotypes (or endophenotypes), based on the assumption that elemental biological phenotypes must underpin schizophrenia and these are likely to have a simpler genetic architecture.<sup>39</sup> Many measures have been evaluated as potential intermediate phenotypes, including most of the biological abnormalities described above. Family studies have suggested that a variety of these abnormalities are indeed familial and likely genetic. They include cognitive impairment, structural and functional imaging abnormalities, altered striatal dopamine release and impaired sensory gating, among others. Some phenotypes may be simpler to model in animals and allow more convincing studies of candidate genes, while others, such as cognition, may not.

## 2.8.2 Results of Linkage Studies

Several dozen linkage studies have been published, none of which are definitive but *in toto* they have implicated several chromosomal regions and genes within those regions. The reasons for the lack of definitive findings are complex and have been described elsewhere. Briefly, linkage approaches are relatively underpowered for complex genetic disorders. Exactly what is inherited is uncertain; for example, it is unclear what psychiatric diagnoses should be included in defining affected family members, only schizophrenia in the narrow sense or all schizophrenia spectrum disorders, such as schizotypal personality disorder. Another knotty issue is that one must prespecify the genetic model (e.g. dominant, recessive). Normally, multiple models are evaluated, incurring a statistical hit for multiplicity. Despite these challenges, a number of studies have been replicated to some degree and have implicated several regions, including 1q32-42, 6p22-24, 8p21-22, 13 q14-32 and 22q11 among others. Data supporting the 1q32 region, and a gene therein, DISC1, are particularly intriguing and come from a large family in Scotland, where most subjects carrying a translocation interfering with DISC1 (*disrupted in schizophrenia-1*) had schizophrenia or a mood disorder.

In the 6p region, implicated genes include dysbindin (DTNBP1) and the major histocompatibility complex. Much work has been done to find specific

mutations or polymorphisms in dysbindin that impact risk, although none has been unequivocally determined. Interestingly, dysbindin appears to play a role in both dopamine and glutamate function.

Regarding other regions, 8p21-22 region harbours neuregulin (NRG1), a large (> 1 Mb) gene with over 45 known splice variants, involved in a wide range of biological effects. Numerous follow-up association studies have implicated SNPs and haplotypes at both ends of this large gene. Evaluating the overall significance level of the follow-up studies is challenging. Supportive follow-up evidence regarding neuregulin has come from studies showing that SNPs in the 5' end may impact splicing and expression in brain tissue from patients with schizophrenia and for a role in a receptor, ErbB4, for one of the many NRG1 isoforms. For 13q14-32, a gene called G72, also known as DAOA, or D-amino acid oxidase activator, appears most promising. Follow-up association studies have provided additional support for this locus, although again the results are not definitive. DAOA has been implicated in glutamate function, suggesting a possible mechanism for its effects on risk. Finally, 22q11 has been found in several linkage studies and is supported by meta analyses. Deletions in this region were previously known to cause velo-cardio-facial syndrome (VCFS), which often includes psychotic symptoms similar to schizophrenia. This region also harbours several popular candidate genes, including COMT, which regulates dopamine neurotransmission, particularly in the prefrontal cortex.

### 2.8.3 Results of Association Studies

Studies of hundreds of specific candidate genes have been published over the last 15 years. Some provide intriguing support for the neurochemical pathways (dopamine, *etc.*) described above as well as genes implicated in linkage studies. For example, these studies tend to suggest that many dopamine-related genes appear to slightly increase risk for schizophrenia. The D<sub>2</sub> receptor is an obvious candidate gene given its role as a target for antipsychotic drugs. There appear to be several functional variants in the D<sub>2</sub> receptor that alter either expression or function, which significantly increase risk in several studies. A second popular candidate gene involved in dopamine neurotransmission is COMT, which has a valine to methionine substitution that produces a substantial impact on enzyme stability and its ability to catabolize synaptic dopamine. The valine allele, which is more active, appears to more rapidly degrade synaptic dopamine, particularly in the PFC, and thereby reduces PFC function and related cognition. Association studies with cognitive- and imaging-based end-points have robustly shown the effects of the val/met allele while the impact of this allele on risk for schizophrenia is less certain. Beyond dopamine, other candidate gene studies have implicated serotonin-related genes, such as 5-HT<sub>2A</sub>, the target of atypical antipsychotic drugs, and genes implicated by linkage studies, such as DISC1, neuregulin, dysbindin. A large body of published work on hundreds of candidate genes is summarized on the SZgene website at [www.schizophreniaforum.org](http://www.schizophreniaforum.org).

The use of chips with hundreds of thousands of SNPs has irrevocably altered the genetic landscape and GWAS studies are now the gold standard for

association studies. Initial GWAS were small and underpowered. One gene surviving rigorous statistical requirements was ZNF804A, a zinc finger protein of uncertain function. A landmark set of three GWAS publications in *Nature* in 2009 found four loci with robust statistical support, including neurogranin (NRGN), the major histocompatibility region on chromosome 6, and an intronic region of transcription factor 4 (TCF4). In addition, these studies reported that a set of hundreds of SNPs, none of which individually met statistical criteria for significance, together contributed to liability for schizophrenia and accounted for at least 30% of the total risk.

A particularly remarkable finding emerging from GWAS studies is the observation that both healthy subjects and patients with schizophrenia have a number of small chromosomal regions that are deleted or duplicated. These so-called structural variants, or copy number variants (CNVs), vary in size, differ between subjects and can interfere with the function of one or more genes or increase the effective copy number, thereby increasing protein levels of specific genes. CNVs in some cases appear to be new mutations, and in others are inherited from parents. Patients with schizophrenia have, on average, an increased number of CNVs. Overall, however, only a small fraction of patients harbour CNVs and the evidence implicating any one specific CNV for impacting risk for schizophrenia is sparse.

## 2.9 Integrated Biological Theories

Attempts to synthesize the diverse array of biological and genetic observations have produced several heuristically valuable theories. Perhaps the most dominant is the neurodevelopmental hypothesis. This theory posits that some abnormality of early brain development, perhaps occurring *in utero*, increases risk for schizophrenia as it emerges in early adulthood. The nature of the abnormality could be an environmental insult, subtle, genetically determined changes in brain “wiring” or connectivity, or some interaction between these two. The delayed onset is often attributed to maturational processes, such as myelination of projections to the PFC or cortical synaptic reorganization that occurs in adolescence. Brain maturation, in this view, enables the full expression of the dysfunctional cortical processes and communication with subcortical structures. A diverse array of studies and clinical findings has been cited in support of this theory. One criticism of the neurodevelopmental hypothesis is that it is not clear to what extent it is falsifiable. Regardless, it has undeniably been influential.

One of the initial findings strongly pointing to a developmental process came in the early 1980s with the identification of clear structural abnormalities and reductions in brain volume. As reviewed above, these deficits predated the onset of the illness, did not appear to progress and, on histological evaluation, were not accompanied by gliosis. The list of biological culprits was very limited, and the proposal that the “lesion” must occur early in development was rapidly embraced.<sup>13</sup> The neurodevelopmental hypothesis received further support by observations of children at high risk years before illness onset, in whom a variety of subtle neurological signs and cognitive deficits were observed. Several influential epidemiological studies reported that the incidence of schizophrenia

was increased roughly 20 years following several unusual environmental insults (e.g. starvation, influenza) impacting pregnant women and their *in utero* offspring. Subsequently, a number of studies have also implicated obstetric complications. Finally, the plausibility that an early cortical lesion could lead to the delayed onset of schizophrenia-related behaviour and dopamine abnormalities was verified by rodent and primate studies (e.g. Tseng *et al.*, 2009).<sup>24</sup>

In contrast to the neurodevelopmental theories, neurodegenerative theories suggest that structural abnormalities are the result of neurodegeneration, which does not result in gliosis. From a clinical perspective, this notion is highly plausible. After the initial onset of schizophrenia, a substantial number of patients show marked deterioration over a 10- to 20-year period in terms of clinical function and severity of both positive and negative symptoms. Response to antipsychotic medication can also diminish. Evidence to support a neurodegenerative process includes MRI studies showing progressive volume loss and cognition studies showing progression in deficits. Not all studies have found these changes, however, particularly progression in cognitive deficits. Furthermore, there are few other data to implicate any known neurodegenerative process. Genetic studies also have not implicated genes in well-known neurodegenerative processes. Nevertheless, it is possible that some aspects of the pathological processes underlying schizophrenia are in some sense progressive.

## 2.10 Conclusions

Schizophrenia is a complex, genetic disorder whose clinical expression is variable. Treatment with atypical antipsychotics can improve positive and, to some extent, negative symptoms, but these medications are only partially effective and there remains a substantial unmet medical need for more effective therapies. Furthermore, cognitive deficits, which appear to account for much of the functional impairment, are not improved with any current therapies. Significant progress has been made over the last 30 years in understanding the biological underpinnings of schizophrenia. Most likely, abnormalities in dopamine are involved. These include increases in pre-synaptic release as well as post-synaptic D<sub>2</sub> density. Other neurotransmitters, including glutamate, GABA and serotonin are also involved. Neuropathological studies suggest subtle alterations in synaptic density may account for the reductions in brain volume observed with MRI. Advances in genetics are slowly revealing some of the genetic underpinnings of these alterations, raising hopes that more effective, rationally designed, therapeutics are on the horizon.

## References

1. T. J. Crow, *Br. Med. J.*, 1980, **280**, 66.
2. N. C. Andreasen, S. A. Olsen, J. W. Dennert and M. R. Smith, *Am. J. Psychiatry*, 1982, **139**, 297.
3. R. W. Buchanan, *Schizophr. Bull.*, 2007, **33**, 1013, Epub 2006 Nov 10.

4. K. H. Nuechterlein, M. F. Green, R. S. Kern, L. E. Baade, D. M. Barch, J. D. Cohen, S. Essock, W. S. Fenton, F. J. Frese 3rd, J. M. Gold, T. Goldberg, R. K. Heaton, R. S. Keefe, H. Kraemer, R. Mesholam-Gately, L. J. Seidman, E. Stover, D. R. Weinberger, A. S. Young, S. Zalcman and S. R. Marder, *Am. J. Psychiatry*, 2008, **165**, 203.
5. J. van Os and S. Kapur, *Lancet*, 2009, **374**, 635.
6. H. Häfner, K. Maurer and A. Riecher-Rössler, *Br. J. Psychiatry*, 1993, **162**, 80.
7. S. W. Woods, J. Addington, K. S. Cadenhead, T. D. Cannon, B. A. Cornblatt, R. Heinssen, D. O. Perkins, L. J. Seidman, M. T. Tsuang, E. F. Walker and T. H. McGlashan, *Schizophr. Bull.*, 2009, **35**, 894.
8. L. J. Seidman, A. J. Giuliano, E. C. Meyer, J. Addington, K. S. Cadenhead, T. D. Cannon, T. H. McGlashan, D. O. Perkins, M. T. Tsuang, E. F. Walker, S. W. Woods, C. E. Bearden, B. K. Christensen, K. Hawkins, R. Heaton, R. S. Keefe, R. Heinssen and B. A. Cornblatt, *Arch. Gen. Psychiatry*, 2010, **67**, 578.
9. S. W. Woods, B. C. Walsh, J. R. Saks and T. H. McGlashan, *Schizophr. Res.*, 2010, **123**, 199.
10. World Health Organization, Geneva, 2001, 28.
11. T. M. Laursen, *Schizophr. Res.*, 2011, **131**, 101.
12. A. Meyer-Lindenberg, *Nature*, 2010, **468**, 194.
13. D. R. Weinberger, *Arch. Gen. Psychiatry*, 1987, **44**, 660.
14. T. R. Insel, *Nature*, 2010, **468**, 187.
15. G. Gründer, H. Hippus and A. Carlsson, *Nat. Rev. Drug Discov.*, 2009, **8**, 197.
16. S. Leucht, C. Corves, D. Arbter, R. R. Engel, C. Li and J. M. Davis, *Lancet*, 2009, **373**, 31.
17. I. Creese, D. R. Burt and S. H. Snyder, *Science*, 1976, **192**, 481.
18. P. Seeman, T. Lee, M. Chau-Wong and K. Wong, *Nature*, 1976, **261**, 717.
19. A. A. Grace, *Neuroscience*, 1991, **41**, 1.
20. P. S. Goldman-Rakic, S. A. Castner, T. H. Svensson, L. J. Siever and G. V. Williams, *Psychopharmacology (Berl.)*, 2004, **174**, 3.
21. M. F. Egan, T. E. Goldberg, B. S. Kolachana, J. H. Callicott, C. M. Mazzanti, R. E. Straub, D. Goldman and D. R. Weinberger, *Proc. Natl Acad. Sci. USA*, 2001, **98**, 6917.
22. M. Akil, B. S. Kolachana, D. A. Rothmond, T. M. Hyde, D. R. Weinberger and J. E. Kleinman, *J. Neurosci.*, 2003, **23**, 2008.
23. M. Slifstein, B. Kolachana, E. H. Simpson, P. Tabares, B. Cheng, M. Duvall, W. G. Frankle, D. R. Weinberger, M. Laruelle and A. Abi-Dargham, *Mol. Psychiatry*, 2008, **13**, 821.
24. K. Y. Tseng, R. A. Chambers and B. K. Lipska, *Behav. Brain Res.*, 2009, **204**, 295.
25. D. C. Javitt and S. R. Zukin, *Am. J. Psychiatry*, 1991, **148**, 1301.
26. B. Moghaddam and D. Javitt, From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment,

- Neuropsychopharmacology*, 2012, Jan; 37(1):4–15. doi: 10.1038/npp.2011.181. Epub 2011 Sep 28.
27. L. M. Rorick-Kehn, B. G. Johnson, K. M. Knitowski, C. R. Salhoff, J. M. Witkin, K. W. Perry, K. I. Griffey, J. P. Tizzano, J. A. Monn, D. L. McKinzie and D. D. Schoepp, *Psychopharmacology (Berl.)*, 2007, **193**, 121.
  28. S. T. Patil, L. Zhang, F. Martenyi, S. L. Lowe, K. A. Jackson, B. V. Andreev, A. S. Avedisova, L. M. Bardenstein, I. Y. Gurovich, M. A. Morozova, S. N. Mosolov, N. G. Neznanov, A. M. Reznik, A. B. Smulevich, V. A. Tochilov, B. G. Johnson, J. A. Monn and D. D. Schoepp, *Nat. Med.*, 2007, **13**, 1102.
  29. M. F. Egan, R. E. Straub, T. E. Goldberg, I. Yakub, J. H. Callicott, A. R. Hariri, V. S. Mattay, A. Bertolino, T. M. Hyde, C. Shannon-Weickert, M. Akil, J. Crook, R. K. Vakkalanka, R. Balkissoon, R. A. Gibbs, J. E. Kleinman and D. R. Weinberger, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 12604.
  30. D. A. Lewis and G. Gonzalez-Burgos, *Nat. Med.*, 2006, **12**, 1016.
  31. H. Y. Meltzer, *Schizophr. Bull.*, 1991, **17**, 263.
  32. J. E. Kleinman, A. J. Law, B. K. Lipska, T. M. Hyde, J. K. Ellis, P. J. Harrison and D. R. Weinberger, *Biol. Psychiatry*, 2011, **69**, 140.
  33. C. Colantuoni, B. K. Lipska, T. Ye, T. M. Hyde, R. Tao, J. T. Leek, E. A. Colantuoni, A. G. Elkahloun, M. M. Herman, D. R. Weinberger and J. E. Kleinman, *Nature*, 2011, **478**, 519.
  34. J. H. Callicott, V. S. Mattay, B. A. Verchinski, S. Marenco, M. F. Egan and D. R. Weinberger, *Am. J. Psychiatry*, 2003, **160**, 2209.
  35. A. Meyer-Lindenberg, J. B. Poline, P. D. Kohn, J. L. Holt, M. F. Egan, D. R. Weinberger and K. F. Berman, *Am. J. Psychiatry*, 2001, **158**, 1809.
  36. A. Meyer-Lindenberg and D. R. Weinberger, *Nat. Rev. Neurosci.*, 2006, **7**, 818.
  37. R. Freedman, L. E. Adler, M. C. Waldo, E. Pachtman and R. D. Franks, *Biol. Psychiatry*, 1983, **18**, 537.
  38. P. F. Sullivan, K. S. Kendler and M. C. Neale, *Arch. Gen. Psychiatry*, 2003, **60**, 1187.
  39. M. F. Egan and T. Cannon, in *Schizophrenia*, ed. D. R. Weinberger and P. J. Harrison, Wiley Blackwell, Chichester, West Sussex, UK, 3rd edn, 2011; [http://www.amazon.com/Schizophrenia-Daniel-R-Weinberger/dp/1405176970#reader\\_1405176970](http://www.amazon.com/Schizophrenia-Daniel-R-Weinberger/dp/1405176970#reader_1405176970).

## CHAPTER 3

# *Monoaminergic Approaches for Treatment of Schizophrenia*

DAVID P. ROTELLA

Margaret and Herman Sokol Professor of Medicinal Chemistry, Department of Chemistry and Biochemistry, Sokol Institute for Pharmaceutical Life Sciences, Montclair State University, Montclair, NJ07043, USA  
Email: rotellad@mail.montclair.edu

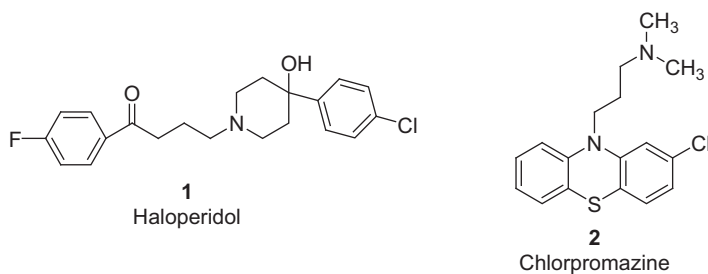
## 3.1 Introduction

### 3.1.1 Overview of Antipsychotic Pharmacotherapy

The discovery and use of haloperidol for the treatment of schizophrenia in the late 1950s and early 1960s (**1**, Figure 3.1) revolutionized the pharmacotherapy of psychiatric disorders.<sup>1</sup> Rapid, beneficial effects on positive symptoms of the disease (*e.g.* hallucinations, delusions, aggressive behaviour) without significant adrenergic or autonomic side-effects at most doses provided physicians and patients with a useful new option to tricyclic antipsychotics such as chlorpromazine (**2**) and related agents. Furthermore, the efficacy of haloperidol at preventing relapse was a particularly noteworthy advantage compared to the tricyclic family of compounds.<sup>2</sup>

Only after these agents were in clinical use was it shown that activity at the molecular level was associated with dopamine receptor antagonism.<sup>3</sup> This observation provided a starting point for the discovery and understanding of the neurochemistry associated with this complex disorder and research in the area continues today. The discovery of distinct dopaminergic signalling





**Figure 3.1** First-generation antipsychotic agents.

pathways and receptors was an early key in the process.<sup>4</sup> For example, four distinct subtypes of dopamine receptors were identified ( $D_1$ – $D_4$ ) and compounds that antagonized receptor function showed antipsychotic activity in humans. Subsequently, it was shown that agents related to **2** elevated cAMP levels ( $D_1$  family receptors), while other compounds such as **1** resulted in reduced cAMP synthesis ( $D_2$ ,  $D_3$ ,  $D_4$  receptors).<sup>5</sup> This helped researchers understand drug efficacy, adverse events and how to evaluate compounds that might display different properties. Inhibition of dopamine signalling in nigrostriatal pathways resulted in Parkinsonian-like adverse effects on muscle movement and control while signalling through the mesolimbic and mesocortical systems exerted the desired antipsychotic activity of dopamine antagonists.

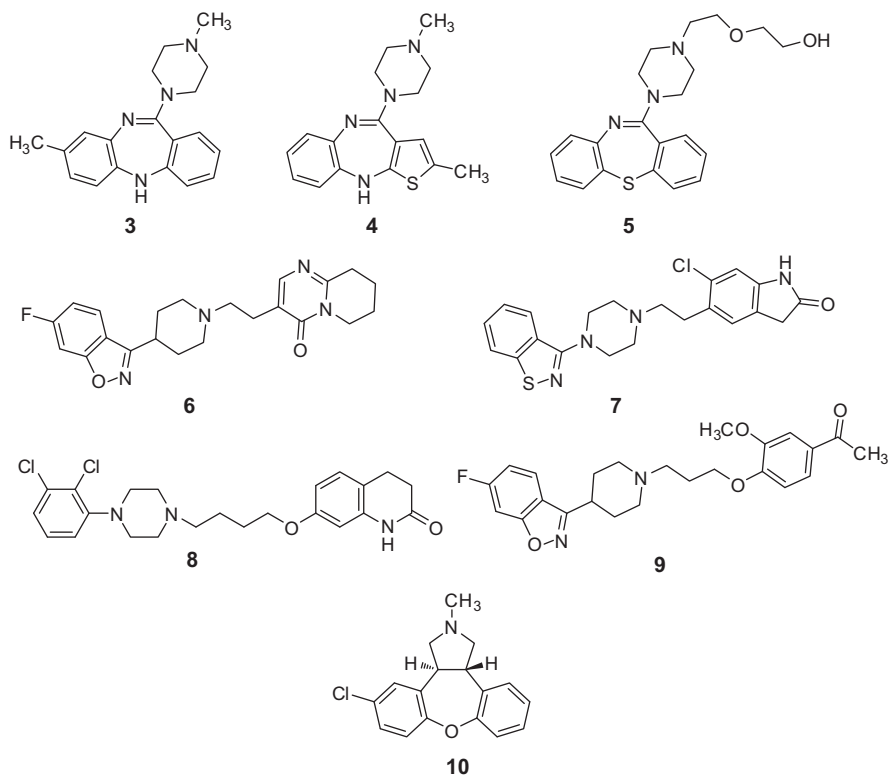
This generation of antipsychotic agents, while effective, displayed important drawbacks and adverse events. Postural hypotension and anticholinergic activity (*e.g.* dry mouth, dizziness and constipation) were commonly observed with tricyclics and extra-pyramidal side-effects, tardive dyskinesia and hyperprolactinemia were frequently seen in patients treated with haloperidol. As a consequence, patients would discontinue drug therapy, leading to a return of symptoms and the inability to function in everyday society. Furthermore, neither structural class demonstrated efficacy in all patients, with up to 60% non-responders, nor did these compounds address the negative (*e.g.* social withdrawal) and/or cognitive symptoms (*e.g.* inability to concentrate, loss of memory) associated with the disease.<sup>1,5</sup> The lack of effective treatment of these issues frequently limits the ability of a patient with schizophrenia to work and engage in otherwise normal personal and societal interactions.

### 3.1.2 Summary of Second-generation Agents

The limitations associated with first-generation antipsychotics provided a strong stimulus for the discovery of compounds that retained the beneficial effects on positive symptomatology with added efficacy for cognitive and negative symptoms, without the adverse events that reduced patient and physician acceptance and adherence to therapy.

Clozapine (**3**, Figure 3.2) represented the first of a now large group of so-called atypical/second-generation antipsychotics and was initially approved for





**Figure 3.2** Second-generation antipsychotic agents.

use in the 1970s. The compound was withdrawn from the market because of the risk of agranulocytosis, but was re-approved in the late 1980s with restrictions and detailed guidelines for use.<sup>6</sup> It was followed by olanzapine (**4**), quetiapine (**5**), risperidone (**6**), ziprasidone (**7**) and more recently by aripiprazole (**8**), iloperidone (**9**) and asenapine (**10**). These drugs have demonstrated efficacy for the treatment of positive symptoms of schizophrenia and for the most part, a substantially reduced risk of extra-pyramidal symptoms and tardive dyskinesia. All of these compounds have activity at one or more dopaminergic receptors and each molecule displays its own unique pharmacology that can be attributed to affinity at a variety of receptors. None of these have yet been conclusively shown to improve negative or cognitive symptoms of the disease.

The clinical efficacy and lack of motor or endocrine side-effects associated with clozapine provided a basis for discovery of potential second-generation antipsychotic compounds. Binding profiles of these compounds (Table 3.1) reveal that with the exception of clozapine and quetiapine, all of these molecules have high affinity for the  $D_2$  receptor and all, except aripiprazole, function as  $D_2$  antagonists; aripiprazole is unique as the only dopamine  $D_2$  partial agonist in this group. This functional distinction contributes to the low

**Table 3.1** Survey of receptor affinity for antipsychotic agents.

	$D_2$	5-HT <sub>2A</sub>	5-HT <sub>2C</sub>	5-HT <sub>1A</sub>	$\alpha 1$	H1	Muscarinic
Haloperidol	1	120	4700	3600	5	61	> 10,000
Clozapine	130	9	17	140	4	2	2
Olanzapine	20	3	10	2100	54	3	5
Quetiapine	180	220	1400	230	15	9	100
Risperidone	2	0.3	10	210	1.4	19	2800
Ziprasidone	3	0.4	1	3	13	47	5100
Aripiprazole	0.3	3	15	2	57	61	> 10,000
Iloperidone	6	6	43	170	0.4	440	> 10,000
Asenapine	1	1	0.3	15	1	9	7000

incidence of motor and endocrine side-effects found with aripiprazole. According to the dopaminergic hypothesis for schizophrenia, hyperactivity in the mesolimbic pathway contributes to delusions and hallucinations (*i.e.* positive symptoms) where antagonism is required for control. The extra-pyramidal and endocrine side-effects result from antagonism in nigrostriatal and tuberfundibular pathways, respectively, where limited signal transduction with a partial agonist would be protective.<sup>7</sup> Addition of 5-HT<sub>2A</sub> antagonist and/or 5-HT<sub>1A</sub> activity as found in all second-generation antipsychotics is another neurochemical approach to attempt to counter the motor issues associated with D<sub>2</sub> antagonism. With respect to 5-HT<sub>2A</sub> affinity, it has been hypothesized that if the K<sub>i</sub> value for 5-HT<sub>2A</sub> receptor affinity is equal to or higher than the D<sub>2</sub> K<sub>i</sub>, the risk of EPS decreases as this ratio increases (*i.e.* K<sub>i</sub> 5-HT<sub>2A</sub>: K<sub>i</sub> D<sub>2</sub> ≥ 1).<sup>8,9</sup>

Some structurally related compounds (*e.g.* **3–5**) have similar affinity for receptors in this panel, while others (**6**, **7** and **9**) display measurable differences at ancillary receptors.<sup>10–12</sup> For example, iloperidone has significantly lower affinity for 5-HT<sub>1A</sub> and H<sub>1</sub> receptors, and higher affinity for  $\alpha 1$  than ziprasidone or risperidone. Since all of these compounds are believed to derive their antipsychotic efficacy primarily by interaction with the D<sub>2</sub> receptor, attempts have been made to correlate *in vivo* receptor occupancy with clinical effect and dopamine receptor-based adverse events. As a result it is commonly accepted that D<sub>2</sub> striatal receptor occupancy between 60 and 80% is necessary for activity and motor side-effects occur when occupancy exceeds 80%.<sup>13,14</sup>

The overall pharmacology associated with activity at the key receptors identified in Table 3.1 leads to expression of a unique adverse event profile for each compound in this group. Detailed evaluation of these differences is beyond the scope of this chapter and the reader is referred to citations herein and other chapters in this book for more information on this subject. In the CATIE trial where the effectiveness and tolerability of risperidone, olanzapine, quetiapine, ziprasidone and perphenazine was evaluated, 75% of patients discontinued pharmacologic therapy because of tolerability issues and second-generation agents were no more efficacious than the first-generation compound perphenazine.<sup>15</sup> This information suggests that the side-effect profiles of all of these agents are unacceptable to the majority of patients. All of these agents are approved for treatment and maintenance of schizophrenia by virtue of their

ability to treat the positive symptoms of the disease. At this point, none of these compounds are widely considered to be efficacious for treatment of negative or cognitive domains. However, there is some evidence that clozapine,<sup>16</sup> aripiprazole,<sup>11</sup> iloperidone<sup>17</sup> and asenapine<sup>12</sup> address one or both of these needs.

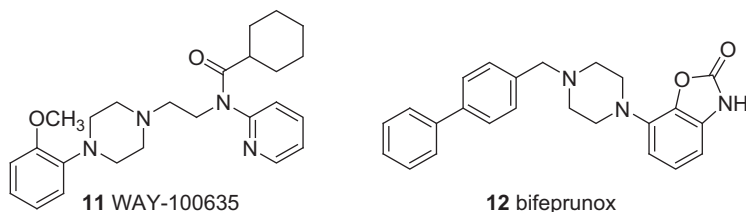
As was noted earlier, the primary dose-limiting side-effects associated with haloperidol are extra-pyramidal symptoms and tardive dyskinesia. These are believed to be associated with D<sub>2</sub> antagonism that is not attenuated because of low affinity for 5-HT<sub>2A</sub> and/or 5-HT<sub>1A</sub> receptors (Table 3.1). Metabolic side-effects (*e.g.* weight gain, dyslipidemia, type 2 diabetes) are now well-recognized effects associated with second-generation antipsychotics including clozapine, olanzapine, quetiapine and risperidone.<sup>18,19</sup> These metabolic issues have been attributed to muscarinic (M<sub>3</sub>), serotonergic (5-HT<sub>2C</sub>) and histamine receptor (H<sub>1</sub>) activity.<sup>20</sup>

Newer agents (aripiprazole, asenapine and iloperidone) do not appear to have these problems, but are not without safety and/or tolerability questions. For example, iloperidone must be titrated over approximately 2 weeks to reduce the incidence of postural hypotension and dizziness (presumably associated with  $\alpha$ 1 activity).<sup>10</sup> Cardiac safety (QT prolongation) associated with asenapine resulted in a “black box” warning and weight gain has been observed in some patients.<sup>12</sup> Aripiprazole has a black box warning for an increased risk of mortality in elderly patients with dementia-related psychosis and increased suicide risk in children and adolescents with major depressive disorder.<sup>21</sup> The remainder of this chapter will focus on serotonergic, D<sub>3</sub>/D<sub>4</sub> receptor ligands and selected combination approaches for the discovery of antipsychotic agents. For a discussion of glutaminergic targets, see Chapter 4 (Morrow, Gilfillan and Neale).

## 3.2 Serotonergic Receptor Targets

### 3.2.1 5-HT<sub>1A</sub> Partial Agonists

The observation that second-generation antipsychotic agents ziprasidone, clozapine and aripiprazole display partial agonist activity *in vivo* and the demonstration that WAY-100635 (**11**, Figure 3.3), a specific 5-HT<sub>1A</sub> antagonist, could reverse antipsychotic induced catalepsy stimulated investigations into the role and potential benefits associated with this receptor.<sup>22</sup> 5-HT<sub>1A</sub>-mediated neurochemical effects in the medial prefrontal cortex have been shown in animal models to be critical for proper attention signalling<sup>23</sup> and aripiprazole is known to augment dopamine and acetylcholine release in the same brain region.<sup>24</sup> The procognitive benefits associated with 5-HT<sub>1A</sub> partial agonists in preclinical models are widely known. For example, bifeprunox (**12**), a mixed D<sub>2</sub> antagonist/5-HT<sub>1A</sub> partial agonist, improved reference memory deficits, working memory and spatial memory in rats with PCP-induced memory loss.<sup>25</sup> Clinical results in humans on the value of 5-HT<sub>1A</sub> activity in enhancing cognition and/or memory provided conflicting results.<sup>26</sup> Sumiyoshi and coworkers provided a recent review on the potential contribution(s) of



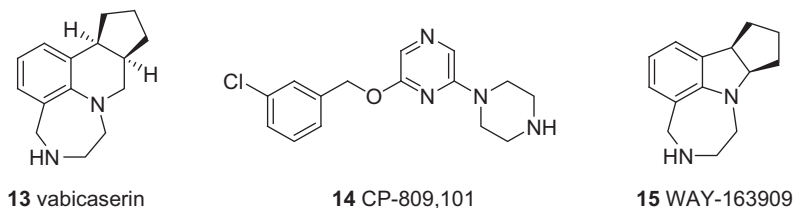
**Figure 3.3** WAY-100635 and bifeprunox.

5-HT<sub>1A</sub> receptors in the pathophysiology of schizophrenia where conflicting data were highlighted along with complicating factors that may contribute to the variability that has been reported.<sup>27</sup>

### 3.2.2 5-HT<sub>2C</sub> Agonists

The 5-HT<sub>2C</sub> receptor is present in brain regions known to be relevant in schizophrenia such as the striatum, nucleus acumbens and cortex.<sup>28</sup> Studies with 5-HT<sub>2C</sub> antagonists elevated synaptic levels of dopamine, an undesirable property in schizophrenia.<sup>29</sup> Conversely, 5-HT<sub>2C</sub> agonists were shown by several groups to reduce dopamine levels in key brain regions, suggesting the potential for antipsychotic activity.<sup>30</sup> Given the adverse metabolic profile associated with some second-generation agents, in particular the antagonist activity and high affinity of clozapine and olanzapine leading to weight gain in many patients and clinical evidence that 5-HT<sub>2C</sub> agonists may result in weight loss,<sup>31</sup> there has been some recent activity in the field related to the discovery of novel antipsychotic agents and one compound, vabicaserin (**13**, Figure 3.4) advanced to phase II studies.<sup>32</sup>

One of the initial reports on the antipsychotic activity of a 5-HT<sub>2C</sub> agonist in animal models of schizophrenia described the properties of CP-809,101, **14** (Figure 3.4).<sup>33</sup> This molecule has K<sub>i</sub> values of 1.6, 6 and 64 nM for human 5-HT<sub>2C</sub>, 5-HT<sub>2A</sub> and 5-HT<sub>2B</sub> receptors, respectively, and is a partial agonist at 5-HT<sub>2B</sub> with an EC<sub>50</sub> value of 65 nM as determined in FLIPR functional assays. As noted above, 5-HT<sub>2A</sub> activity is likely to be beneficial; however, 5-HT<sub>2B</sub> agonist activity is associated with cardiac valvulopathy and must be avoided.<sup>34</sup> In conditioned avoidance responding, **14** showed dose-dependent activity with an ED<sub>50</sub> of approximately 5 mg/kg sc. This activity could be antagonized by concurrent administration of a selective 5-HT<sub>2C</sub> antagonist. CP-809,101 demonstrated activity in a novel object recognition screen reflective of procognitive potential, PCP and d-amphetamine induced hyperactivity and reversed apomorphine induced deficits in a prepulse inhibition model. The compound did not show evidence of catalepsy up to 56 mg/kg. Overall, this profile suggested a low incidence of extra-pyramidal side-effects with properties similar to a second-generation antipsychotic.

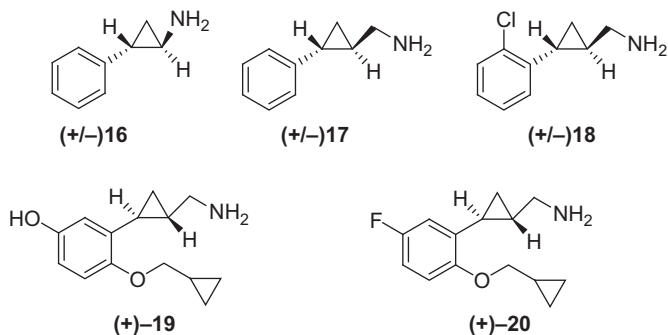


**Figure 3.4** 5HT<sub>2C</sub> agonists.

Marquis and coworkers at Wyeth reported the preclinical characterization of WAY-163909 (**15**), a compound structurally related to vabicaserin.<sup>30</sup> Like **14**, **15** was active at comparable doses in conditioned avoidance responding and hyperactivity models and did not induce catalepsy. The desired *in vivo* effects in antipsychotic models were antagonized by a 5-HT<sub>2C</sub> antagonist. Neurochemically, WAY-163909 selectively decreased extra-cellular levels of dopamine in the nucleus acumbens and had no effect on dopamine levels in the striatum. *In vivo* acute and chronic (up to 21 days) electrophysiology studies at doses up to 10 mg/kg ip demonstrated that **15** decreased spontaneous firing of dopaminergic neurons in the ventral tegmental area but not in the substantia nigra. This neurochemical profile supported the behavioural results to provide a stronger basis to validate the 5-HT<sub>2C</sub> receptor as a potential target for schizophrenia.

This comprehensive characterization supported the antipsychotic potential of a 5-HT<sub>2C</sub> agonist and contributed to the discovery of vabicaserin (SCA-136), **13**.<sup>35</sup> This diazepine analogue of WAY-163909 showed K<sub>i</sub> values of 3, 14 and 112 nM for binding to human 5-HT<sub>2C</sub>, 5-HT<sub>2B</sub> and 5-HT<sub>1A</sub> receptors, respectively. More than 50-fold binding selectivity was found *versus* other serotonin, dopamine and  $\alpha$ 1 receptors. Functionally, **13** was a full 5-HT<sub>2C</sub> agonist using a FLIPR assay in CHO cells expressing the recombinant human receptor. Potency and efficacy at 5-HT<sub>2B</sub> receptors was dependent on the level of recombinant receptor expression. In a system where the 5-HT<sub>2B</sub> receptor is naturally expressed (rat stomach fundus), antagonist activity was demonstrated using serotonin as the agonist ligand. Similar activity was observed in human colon tissue preparations. Weak and variable agonist effects could be seen in isolated superior mesenteric artery preparations. To date, neither the preclinical *in vivo* activity nor the result of human trials of vabicaserin have been published and its clinical status is unknown following completion of phase II studies in patients with schizophrenia.

More recent medicinal chemistry results in this area on a class of cyclopropylalkylamino derivatives were disclosed by Kozikowski and coworkers. In the first of two reports, using the monoamine oxidase inhibitor tranylcypromine **16** (Figure 3.5) as a lead, it was found that homologation to **17** resulted in a potent, full 5-HT<sub>2C</sub> agonist (EC<sub>50</sub> 13 nM) with moderate selectivity *versus* the 5-HT<sub>2B</sub> receptor.<sup>36</sup> The *trans* isomer **17** was more potent than the corresponding *cis* isomer. Resolution of the racemic mixture revealed that the (S,S)-(+ ) isomer of



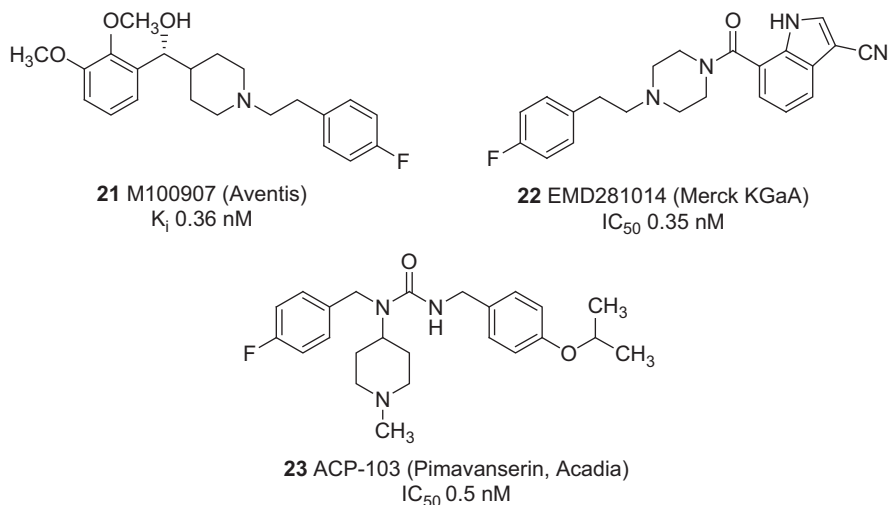
**Figure 3.5** Cyclopropylalkylamino 5HT<sub>2C</sub> agonists.

**17** was substantially more potent than the (R,R)-(-) isomer. Methylation of the primary amine in **17** reduced 5-HT<sub>2C</sub> affinity. Halogenation of **17** (mono- or poly-) in general had no effect on selectivity *versus* 5-HT<sub>2B</sub> and provided variable effects on 5-HT<sub>2C</sub> affinity, with 2-substitution preferred, *e.g.* **18**. All of the halogenated derivatives proved to be full agonists in functional receptor assays. In a second study, optically pure (+)-isomers of **19** and **20** were prepared and studied.<sup>37</sup> These two compounds were selected because they showed full 5-HT<sub>2C</sub> agonist efficacy similar to vabicaserin and functioned as 5-HT<sub>2B</sub> antagonists in an HEK293 cell line using a calcium flux assay. In a PCP-induced PPI assay, both compounds were active at 5 and 10 mg/kg ip, with a profile similar to vabicaserin at the same doses.

### 3.2.3 5-HT<sub>2A</sub> Antagonists

Interest in the 5-HT<sub>2A</sub> receptor as a potential target for treatment of schizophrenia arose in part from the observation that lysergic acid diethylamide exhibited potent hallucinogenic properties *in vivo* and this activity was associated with potent 5-HT<sub>2A</sub> affinity.<sup>38</sup> The potential for modulating monoamine neurotransmission (analogous to the role of the 5-HT<sub>2C</sub> receptor) in brain regions associated with schizophrenia<sup>39,40</sup> and the neurochemical and *in vivo* profiles of second-generation antipsychotics created a strong stimulus to investigate this target.

Mice lacking the dopamine transporter (DAT) display behavioural characteristics associated with hyperdopaminergic activity. When administered to DAT knockout mice, the selective 5-HT<sub>2A</sub> antagonist M100907 (**21**, Figure 3.6) reversed prepulse inhibition and locomotor deficits at doses of 0.3 and 1 mg/kg.<sup>41</sup> This activity profile is similar to that observed with second-generation antipsychotic agents and provided behavioural support for the neurochemical hypotheses cited above. A recent study by Gozzi *et al.* revealed that M100907 exerts these actions in the prefrontal cortex *via* glutamate-mediated neurotransmission.<sup>42</sup>



**Figure 3.6** 5HT<sub>2A</sub> antagonists.

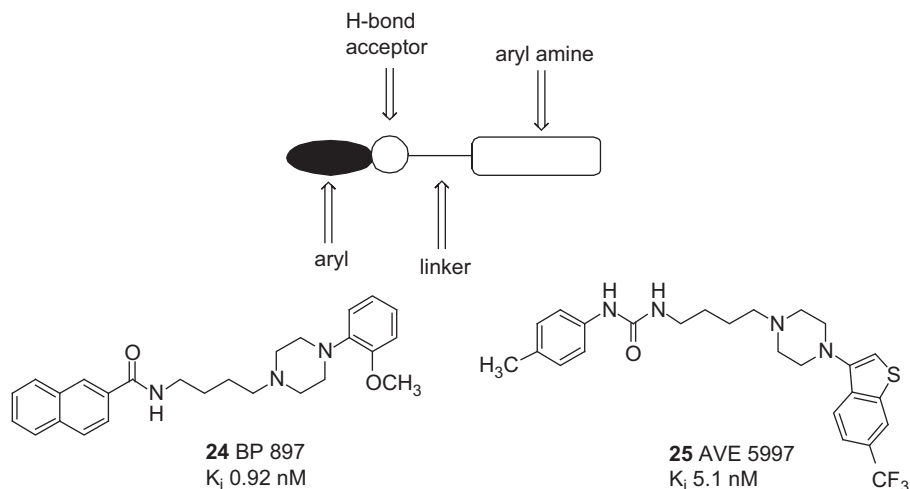
The procognitive properties associated with 5-HT<sub>2A</sub> antagonism were illustrated by EMD 281014, **22**, in young and aged rhesus monkeys.<sup>43</sup> Animals were trained to perform a computer-assisted delayed matching to sample task to match colours. Oral administration of **22** over a dose range of 0.1–10 mg/kg revealed that the drug showed memory enhancing effects in both age groups. Younger monkeys showed positive effects only at 10 mg/kg, while the older group had positive results at 3 and 10 mg/kg. No acute adverse events were notable during the course of drug administration.

ACP-103, **23**, was shown by Gardell and coworkers to improve the efficacy of haloperidol and risperidone in hyperactivity models of schizophrenia, while reducing catalepsy and hyperprolactinemia.<sup>44</sup> This was reflected in left shifts in dose response curves using a fixed dose of **23** (0.03 mg/kg) and various doses of each antipsychotic. Reduced serum prolactin levels were measured in rats treated with all dose combinations of ACP-103 and either haloperidol or risperidone. The positive effects on catalepsy were observed at higher doses of ACP-103 (~1–3 mg/kg), however the beneficial effects were dose-dependently observed with both antipsychotics.

## 3.3 Recent Results in Dopaminergic Receptors

### 3.3.1 D<sub>3</sub> Receptors

The dopamine D<sub>3</sub> receptor was first cloned and characterized in 1990 by Sokoloff and coworkers.<sup>45</sup> D<sub>3</sub> receptors are found primarily in limbic regions of the CNS. This distribution suggests potential use in treatment of schizophrenia and other CNS disorders, including drug addiction.<sup>46,47</sup> A recent review



**Figure 3.7** D<sub>3</sub> pharmacophore model and early compounds.

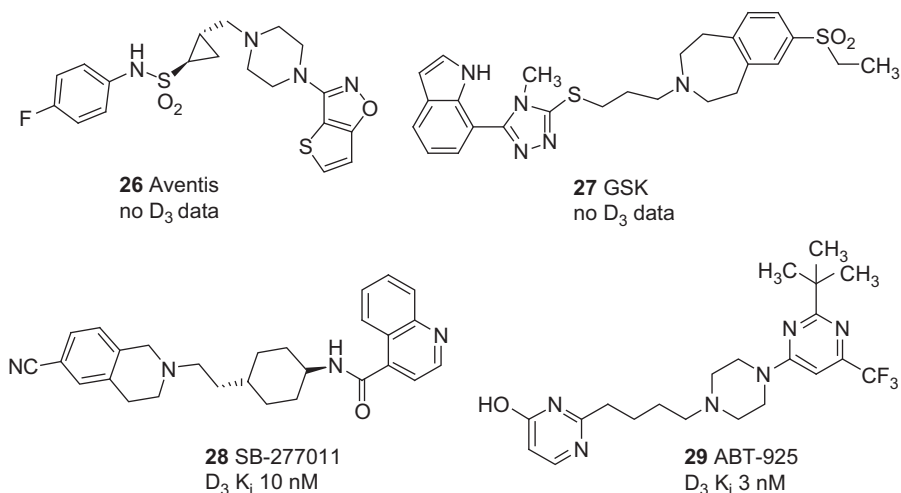
summarizes work carried out in the field in the early years of this decade to identify D<sub>3</sub> ligands and presents a pharmacophore model that was developed as a result of these investigations. The model (Figure 3.7) includes features commonly found in CNS active agents, namely lipophilic amines linked to an aryl moiety.<sup>48</sup> An aryl piperazine fragment (*e.g.* BP 897, **24**) occurs in several structures and an amide is often present as a hydrogen bond acceptor, although a urea as in AVE 5997 (**25**) can also be used.

Many of the leads in Figure 3.7 displayed excellent D<sub>3</sub> affinity, but had unfavourable pharmaceutical properties and/or receptor selectivity profiles. Attempts to address these problems resulted in structures such as those shown in Figure 3.8. These D<sub>3</sub> antagonists employ other hydrogen bond acceptors, *e.g.* **26** and **27**, as well as conformationally constrained linkers such as that found in SB-277011 (**28**)<sup>49</sup> and more hydrophilic aryl moieties such as ABT-925 (**29**).<sup>50</sup> These compounds show improved D<sub>3</sub> selectivity, a feature that was essential to address the hypothesis that D<sub>2</sub> activity contributed to some of the adverse events associated with first- and second-generation antipsychotic agents.<sup>48</sup>

ABT-925 advanced to clinical development and the results of a phase II trial in patients with acute schizophrenia were recently reported.<sup>51</sup> The compound was studied in 155 patients in a double-blind randomized, placebo-controlled study over six weeks at doses of 50 and 150 mg once daily. Neither dose of **29** was efficacious, as measured by behavioural evaluation according to the Positive and Negative Syndrome Scale. The compound was well tolerated at both doses and evidence from a PET study in healthy volunteers suggested<sup>52</sup> that a dose of at least 450 mg was needed to produce sufficient receptor occupancy to exert a pharmacologic effect.

S33138 (**30**, Figure 3.8) was shown to have 40-fold higher affinity for D<sub>3</sub> receptors compared to D<sub>2</sub> and even better selectivity for other dopaminergic

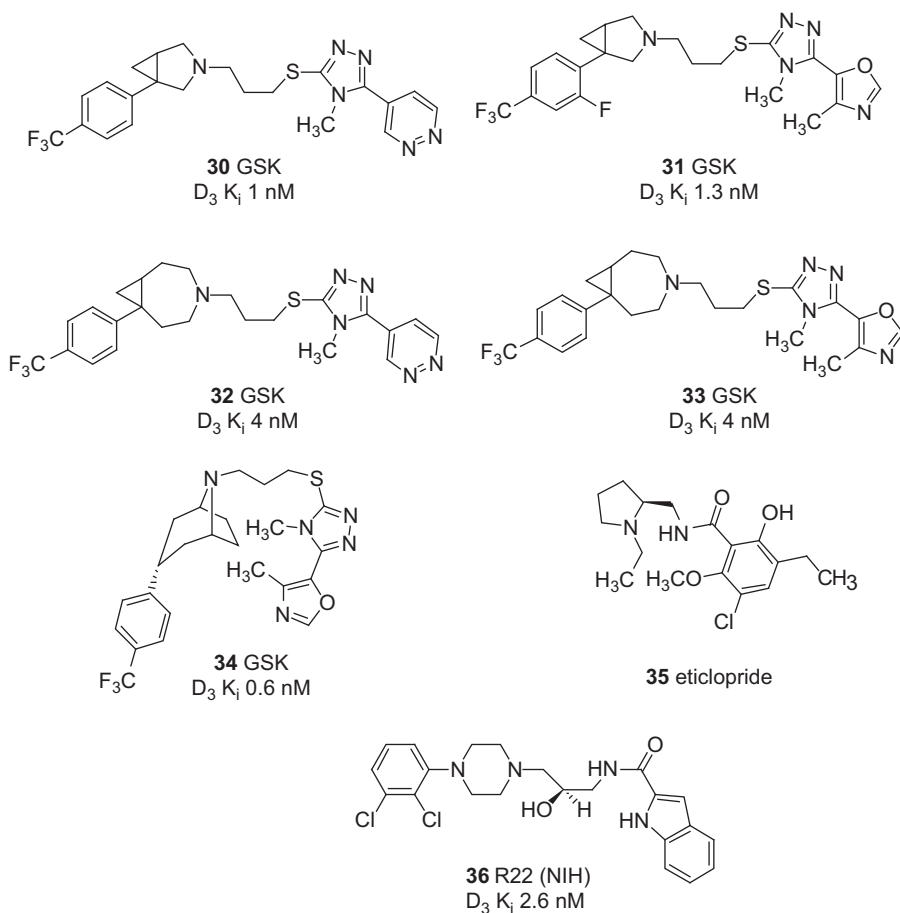




**Figure 3.8** Examples of D<sub>3</sub> antagonists.

receptors.<sup>53</sup> Against a panel of serotonergic and adrenergic receptors good ( $50\times$ ) to excellent ( $>1,000$ ) selectivity was demonstrated. Functional studies showed the compound was a full antagonist at D<sub>3</sub> receptors. Subsequent *in vivo* experiments demonstrated that **30** could restore cognitive function in rodents and primates in a dose-dependent manner.<sup>54</sup> In rats, S33138 blocked impairment of novel object recognition (NOR) and social novelty discrimination (SNR) in rats when administered subcutaneously. Doses ranged from a lower limit of 0.01 mg/kg (NOR) to 2.5 mg/kg (SNR). Rhesus monkeys were trained then treated with MPTP, memory deficits were reversed and accuracy was improved at doses of 0.04 and 0.16 mg/kg sc. In aged monkeys, improved task accuracies were observed following doses of 0.16 and 0.63 mg/kg po. These data suggest the compound has potential to address cognitive deficits in schizophrenia.

Two recent papers from GSK reported SAR studies of D<sub>3</sub> antagonists with an azabicyclo[3.1.0] linker. In the first report<sup>55</sup> using the aryl triazolo-thio fragment found in **27**, an aryl-azabicyclo[3.1.0] building block was employed as an alternate to the azepine ring in **27** because this unit furnished a more attractive pharmaceutical property profile (*e.g.* TPSA, cLogD) compared to the benzazepine moiety in **27**. A wide range of aryl substituents was evaluated and both electron-donating and -withdrawing groups furnished potent D<sub>3</sub> antagonists ( $K_i \leq 10$  nM) with excellent selectivity *versus* D<sub>2</sub> receptors and low hERG affinity. Selected compounds in this group were studied in greater detail (*e.g.* **30** and **31**, Figure 3.9) for receptor selectivity and pharmaceutical properties including cardiac safety as measured by QTc prolongation in anaesthetized guinea pigs. The derivatives in Figure 3.9 had greater than 100-fold selectivity for other receptors and enzymes in a 100-target panel. Furthermore, they demonstrated acceptable P450 inhibition, clearance and oral bioavailability (data not shown). Anaesthetized guinea pigs received up to 15 mg/kg by



**Figure 3.9** Azabicyclo-based  $D_3$  antagonists.

intravenous infusion and showed no evidence for QTc prolongation. These compounds also showed activity in animal models of drug addiction following intra-peritoneal administration.

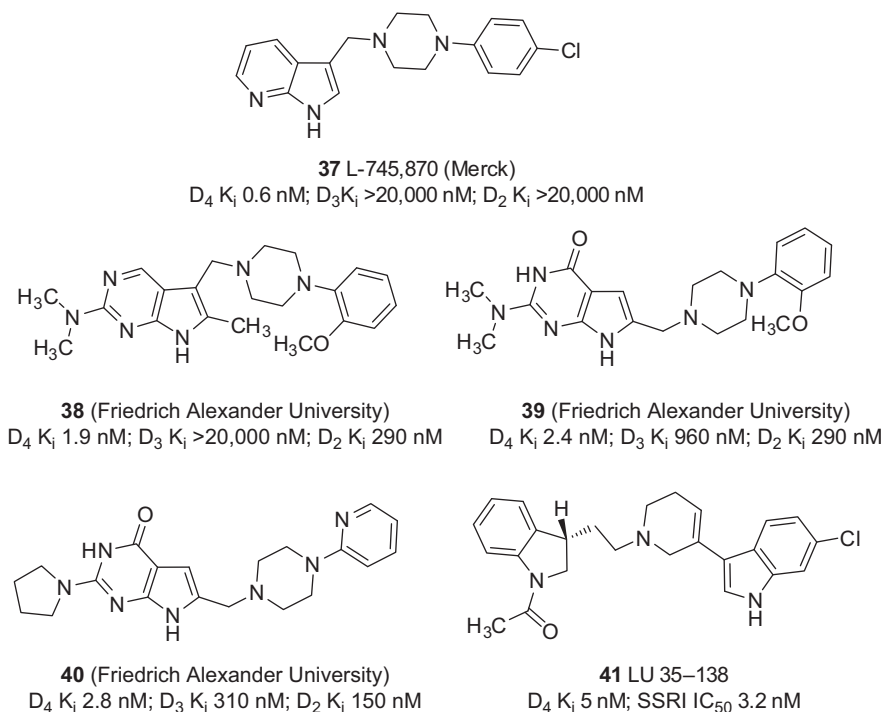
The second paper in this series<sup>56</sup> reported structure activity studies on variations on the azabicyclo[3.1.0] region of the molecule. Substitution of a piperidine, aminocyclopentane or more complex azaspirocycles for pyrrolidine furnished less potent analogues. However, an azabicyclo[4.1.0] framework as in **32** or **33**, while slightly less potent, furnished equally good dopamine receptor and hERG selectivity. A tropane derivative **34** provided  $D_3$  potency comparable to **30** or **31** with low hERG affinity.

Structure-based design of  $D_3$  ligands may be aided by the report from Chien, Liu and coworkers, which provided a crystal structure using the  $D_2/D_3$  antagonist eticlopride **35** (Figure 3.9).<sup>57</sup> This structure was obtained using a point mutation in the transmembrane domain and by replacing most of the

third cytoplasmic loop with T4-lysozyme. Purification of the receptor with **35** bound provided the most thermostable complex. Important interactions include an ionic association between the pyrrolidine nitrogen of **35** and aspartate 110 and a number of hydrophobic interactions between the substituted phenyl ring and residues on the receptor. This structure highlighted potential interactions to furnish  $D_3$ -selective ligands, especially those that take advantage of a binding pocket in an extra-cellular loop on the receptor. This was demonstrated by modelling the association of the  $D_3$ -selective ligand R-22<sup>58</sup> (**36**, Figure 3.9) with the receptor. The model suggests the indole portion of the molecule extends into this pocket, which is formed by two extra-cellular loops and helices I, II and VII on the  $D_3$  receptor. These regions in the  $D_2$  receptor are different enough in structure that this region does not accommodate **36**. It will be interesting if this hypothesis can be confirmed experimentally by subsequent experiments.

### 3.3.2 $D_4$ Receptors

Comparatively little new work has been carried out on the discovery of new  $D_4$  antagonists for treatment of schizophrenia in the last several years following the clinical failure reported with L-745,870 (**37**, Figure 3.10) as a potential antipsychotic agent.<sup>59</sup>



**Figure 3.10**  $D_4$  antagonists.

Based on the highly D<sub>4</sub>-selective Merck lead, L-745,870, Linz and coworkers prepared a series of Mannich base derivatives using other heterocycles.<sup>60</sup> Structure activity studies revealed that the three analogues shown in Figure 3.10 (**38–40**) displayed excellent D<sub>4</sub> affinity and in some cases selectivity comparable to **37**. When the dopaminergic affinity profiles of these molecules were compared to clozapine (D<sub>4</sub> K<sub>i</sub> 16 nM, D<sub>3</sub> K<sub>i</sub> 960 nM, D<sub>2</sub> K<sub>i</sub> 28 nM), each of them was superior in terms of the D<sub>4</sub>/D<sub>2</sub> ratio. However, compared to L-745,870, which is effectively inactive at other dopamine receptors, this series displays measurable D<sub>2</sub> and D<sub>3</sub> affinity.

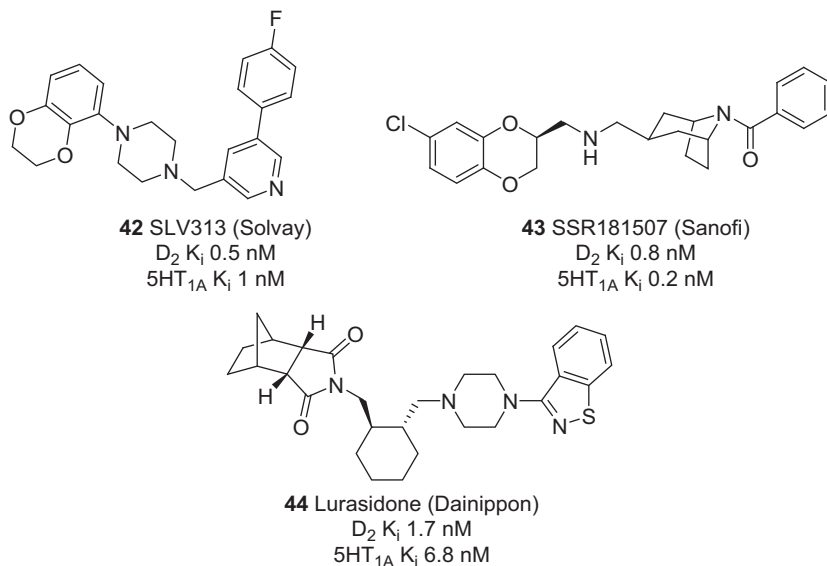
Researchers at Lundbeck reported the discovery of LU 35-138 (**41**),<sup>61</sup> a compound that has good D<sub>4</sub> affinity (K<sub>i</sub> 5 nM) and functions as a full antagonist combined with similarly potent serotonin receptor uptake inhibition (IC<sub>50</sub> 3 nM). This molecule retains some  $\alpha$ 1 and D<sub>2</sub> receptor activity (*ca.* 10–15  $\times$  greater than D<sub>4</sub>) and displays improved serotonergic receptor selectivity compared to clozapine, haloperidol, olanzapine and risperidone. In hyperlocomotion assays, **41** had ED<sub>50</sub>s of 4 mg/kg sc in rats (amphetamine-induced) and 13 mg/kg sc in mice (PCP-induced). LU 35-138 did not cause catalepsy in rats, did not degrade spatial memory in a water maze assay in rats and did not result in dystonia in primates. This profile suggested the potential for antipsychotic activity without negative extra-pyramidal or cognitive side-effects.

## 3.4 Selective Poly-target Approaches

### 3.4.1 D<sub>2</sub>/5-HT<sub>1A</sub>

As noted in Section 3.2, combining dopamine D<sub>2</sub> antagonism with 5-HT<sub>1A</sub> partial agonist activity provides compounds with potentially attractive pharmacologic profiles as antipsychotic agents. However, approved second-generation molecules with these properties also have other activity that is undesirable. As a result, considerable attention has been focused on the discovery of compounds with more selective receptor binding profiles in an attempt to retain the beneficial aspects of this dual-target approach and reduce the side-effect potential.

A direct comparison between this dual target approach and first- or second-generation antipsychotics was reported by Bardin and coworkers.<sup>62</sup> This group used the D<sub>2</sub> antagonist/5-HT<sub>1A</sub> partial agonists bifeprunox (**12**, Figure 3.3), SLV313, **42**<sup>63</sup> (Figure 3.11) and SSR181507, **43**<sup>64</sup> and compared their activity in animal models of schizophrenia as well as side-effect potential (as measured by catalepsy and extra-pyramidal effects) with haloperidol (**1**), ziprasidone (**7**), risperidone (**6**), aripiprazole (**8**) and olanzapine (**4**). All of these molecules were active in antipsychotic models with ED<sub>50</sub> values in conditioned avoidance responding that ranged from 0.03 mg/kg (**12**) to 3.2 mg/kg (**8**). SLV313 and SSR181507 had ED<sub>50</sub> values of 0.25 mg/kg and 0.58 mg/kg, respectively. In catalepsy studies, **12**, **42** and **43** along with aripiprazole all had ED<sub>50</sub> values greater than 40 mg/kg, while haloperidol showed the greatest potential for EPS



**Figure 3.11**  $D_2$  antagonist/ $5HT_{1A}$  partial agonist compounds.

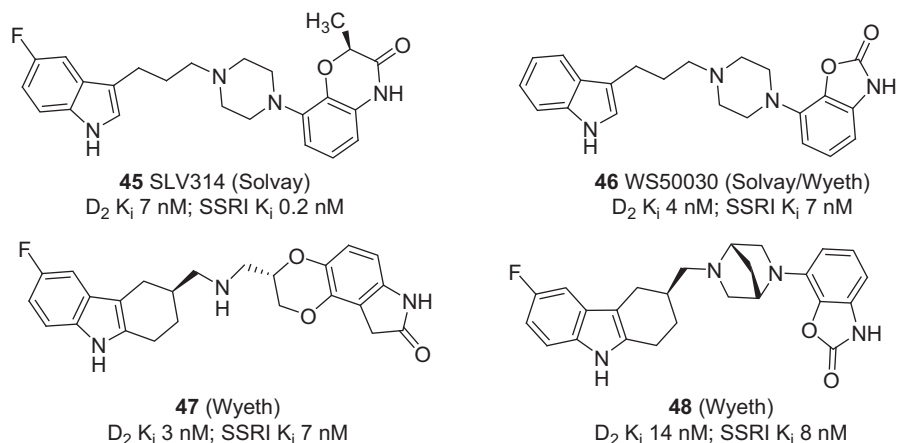
with an  $ED_{50}$  of 0.3 mg/kg, followed by risperidone (1.8 mg/kg) and olanzapine (7.1 mg/kg). These results suggest an inverse relationship between  $5-HT_{1A}$  receptor affinity and cataleptogenic potential as one measure of side-effect potential, while activity in conditioned avoidance corresponded with  $D_2$  affinity.

Lurasidone<sup>65</sup> (**44**) is another  $D_2$  antagonist/partial  $5-HT_{1A}$  agonist with a more complex receptor binding profile that was recently reported to demonstrate antipsychotic activity in preclinical models (conditioned avoidance  $ED_{50}$  6 mg/kg) with low potential for extra-pyramidal effects (catalepsy  $ED_{50}$  > 1,000 mg/kg). This molecule also has potent activity at  $5-HT_7$ ,  $5-HT_{2A}$  and  $\alpha_{2C}$  receptors with weak affinity at muscarinic,  $H_1$ ,  $5-HT_{2C}$  and  $\alpha_1$  sites.

### 3.4.2 $D_2$ /Serotonin Reuptake Inhibition

The rationale for combining these two properties in a single molecule is based on clinical observations suggesting that serotonin reuptake inhibition (SRI) offers the potential to treat negative symptoms of schizophrenia<sup>66,67</sup> and may also be useful to address co-morbid depression that accompanies the disease.

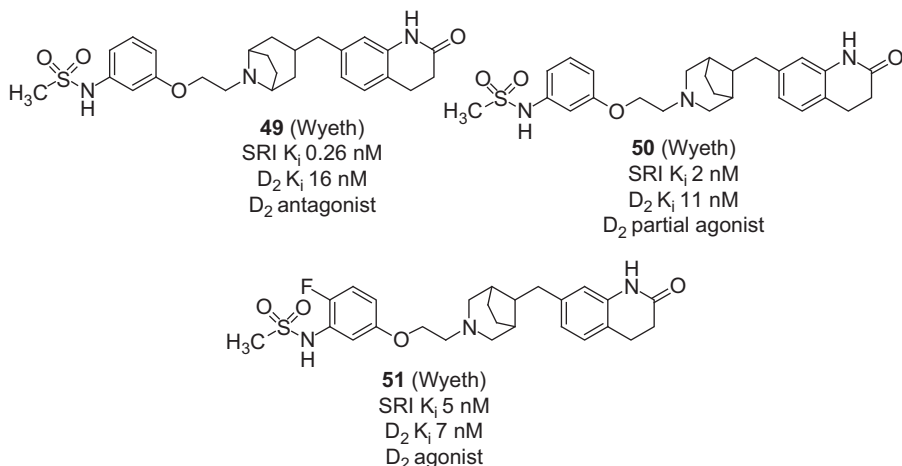
Smid and colleagues<sup>68</sup> showed that a combined  $D_2$  antagonist/SRI such as SLV314 **45** (Figure 3.12) had efficacy in antipsychotic models with evidence for serotonin reuptake inhibition in a serotonin syndrome model. This molecule had  $K_i$  values of 7 nM and 0.2 nM, respectively, at the  $D_2$  and SRI targets and functioned as a complete  $D_2$  antagonist. SLV314 had  $ED_{50}$ s of 0.08 mg/kg and 0.15 mg/kg orally, respectively, in apomorphine-induced climbing and 5-HTP serotonin potentiation models. Building on this observation and with the



**Figure 3.12** SLV314 D<sub>2</sub> antagonist/SRI and WS-50030 D<sub>2</sub> partial agonist/SRI.

knowledge that D<sub>2</sub> partial agonist activity (as in aripiprazole) could provide an improved side-effect profile; Brennan and coworkers showed that WS-50030 (**46**) (D<sub>2</sub> K<sub>i</sub> 4 nM, SRI K<sub>i</sub> 7 nM) had a profile similar to aripiprazole in conditioned avoidance responding (both compounds were active at 10 mg/kg orally) and apomorphine induced climbing at doses that induced minimal catalepsy.<sup>69</sup> Chronic microdialysis experiments following a 10 mg/kg oral dose daily for 21 days showed that **46** elevated serotonin levels in the rat medial prefrontal cortex as expected for an SRI compound. Partial dopamine D<sub>2</sub> agonist activity was demonstrated in the 6-OH DA turning rat model, where a 10 mg/kg oral dose produced a profile like aripiprazole. The potential antidepressant effect of **46** was highlighted in a chronic olfactory bulbectomy model where the compound reduced hyperactivity in rats over a dose range of 3–10 mg/kg after 14 days of dosing.

Two other reports describe additional chemotypes with partial D<sub>2</sub> agonist/SRI activity. Rotella *et al.* used alkyl amino carbazole SRI building blocks with benzodioxolanyl lactam and phenylloxazolone D<sub>2</sub> ligands to prepare a series of dual-acting molecules.<sup>70</sup> In an attempt to improve receptor selectivity, emphasis was directed toward variations in the linker that connected the SRI and D<sub>2</sub> fragments. This series of compounds identified derivatives with and without 5-HT<sub>1A</sub> affinity, *e.g.* **47** and **48**. Benzodioxane derivative **47** had a D<sub>2</sub> K<sub>i</sub> of 3 nM, an SRI K<sub>i</sub> of 7 nM and a 5-HT<sub>1A</sub> K<sub>i</sub> of 2 nM with partial D<sub>2</sub> agonist and full 5-HT<sub>1A</sub> agonist functional activity. Phenylloxazolone analogue **48** had K<sub>i</sub> values of 14, 8 and 1800 nM at D<sub>2</sub>, SRI and 5-HT<sub>1A</sub> receptors, respectively. When these compounds were tested *in vivo*, in apomorphine-induced climbing, **48** showed a profile similar to clozapine and was inactive in a 5-HTP serotonin model (30 mg/kg ip) with measurable catalepsy in mice at 10 mg/kg. Likewise, **47** was inactive in the 5-HTP screen. These observations were hypothesized to be attributable to the lack of substantial separation between the primary targets and the  $\alpha$ 1 receptor (65 and 23 nM, respectively, for **48** and **47**).



**Figure 3.13**  $D_2$  partial agonists/SRI compounds.

In an attempt to improve receptor selectivity, this group reported *in vitro* results with other SRI and  $D_2$  building blocks.<sup>71</sup> In particular, 7-substituted benzolactams (similar to aripiprazole) were investigated based on the hypothesis that this might improve serotonin receptor selectivity. Piperidine-based linkers were highlighted in this manuscript following the observation that alkyl piperazine linkers provided low SRI affinity with phenylloxazolone  $D_2$  ligands ( $K_i > 90$  nM). Bridged piperidines demonstrated distinct  $D_2$  functional activity, with an unexpected difference in affinity at SRI and  $D_2$  receptors (Figure 3.13). The 8-aza-isomer **49** was an antagonist in a GTP $\gamma$ S assay, while the 3-aza-isomer **50** functioned as a partial agonist. Fluorosulfonamide **51** had similar affinity and receptor selectivity to **49** and **50** but functioned as a  $D_2$  agonist. In this series, an aryl sulfonamide  $D_2$  fragment in combination with the bridged piperidine linker proved to be essential for improved  $\alpha 1$  receptor selectivity.

### 3.5 Summary and Outlook

Targeting monoaminergic receptors is a proven approach for the treatment of schizophrenia. The accumulated knowledge and experience with compounds such as haloperidol, clozapine and aripiprazole indicate it is important to interact with more than one in order to provide an efficacious and acceptable antipsychotic (“magic shotgun” hypothesis).<sup>72</sup> The precise choice of receptors remains an open question and, from a medicinal chemistry perspective, a substantial challenge. Incorporation of multiple receptor pharmacophores in a brain penetrant molecule that has appropriate pharmaceutical and physical properties is a problem that has not been adequately solved.

In spite of these issues, or perhaps because of them, the discovery of safe and effective antipsychotic agents remains an extremely active area of research. The well-recognized need to address more than one symptom domain in

schizophrenia is a driving force for preclinical and clinical models that suggest and measure efficacy. This unmet need is part of the driving force associated with the development of combination therapy approaches as well as dual-/multi-target drug-discovery efforts. As understandings of the neurochemical pathways that influence these distinct behavioural traits develop, researchers can define options for pharmacologic intervention. Beyond this symptomatic approach, ongoing efforts to identify contributors to the pathology associated with schizophrenia have the potential to reveal disease-modifying targets for this crippling disorder.

## References

1. E. C. Settle, Jr and F. J. Ayd, Jr, *J. Clin. Psychiatry*, 1983, **44**, 440.
2. J. M. Kane, *J. Clin. Psychiatry*, 1989, **50**, 322.
3. A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol.*, 1963, **20**, 163.
4. U. Ungerstedt, *Acta Physiol. Scand. Suppl.*, 1971, **367**, 1.
5. For an excellent recent summary of these studies, see R. B. Mailman and V. Murthy, *Curr. Pharm. Design*, 2010, **16**, 488.
6. J. Crilly, *History of Psychiatry*, 2007, **18**, 39.
7. D. Bishara and D. Taylor, *Drugs*, 2008, **16**, 2269.
8. H. Y. Meltzer, *Neuropsychopharmacology*, 1999, **21**, 106.
9. S. Kongsamut, J. E. Roehr, J. Cai, H. B. Hartman, P. Weissensee, L. L. Kerman, L. Tang and A. Sandrasagra, *Eur. J. Pharmacol.*, 1996, **317**, 417.
10. B. L. Crabtree and J. Montgomery, *Clin. Therapeutics*, 2011, **33**, 330.
11. S. Gupta and P. Masand, *Ann. Clin. Psychiatry*, 2004, **16**, 155.
12. A. Minassian and J. W. Young, *Expert Opin. Pharmacother.*, 2010, **11**, 2107.
13. J. Arndt and T. Skarsfeldt, *Neuropsychopharmacology*, 1998, **18**, 63.
14. S. Nyberg, L. Farde, L. Eriksson, C. Halldin and B. Eriksson, *Psychopharmacology*, 1993, **110**, 265.
15. J. A. Lieberman, T. S. Stroup, J. P. McEvoy, M. S. Swartz, R. A. Rosenheck, D. O. Perkins, R. S. Keefe, S. M. Davis, C. E. Davis, B. D. Lebowitz, J. Severe and J. K. Hsiao, *New Engl. J. Med.*, 2005, **353**, 1209.
16. Summarized in: R. D. Porsolt, P. C. Moser and V. Castagne, *J. Pharmacol. Exp. Ther.*, 2010, **333**, 632.
17. J. Marino and J. Caballero, *Ann. Pharmacother.*, 2010, **44**, 863.
18. P. Pramyothin and L. Khaodhiar, *Curr. Opin. Endocrinol. Diabetes Obes.*, 2010, **17**, 460.
19. R. R. Girgis, J. A. Javitch and J. A. Lieberman, *Mol. Psychiatry*, 2008, **13**, 918.
20. H. A. Nasrallah, *Mol. Psychiatry*, 2008, **13**, 27.
21. Aripiprazole prescribing information found at [www.abilify.com](http://www.abilify.com); for additional detail see I. Kohen, P. E. Lester and S. Lam, *Neuropsychiatr. Dis. Treat.*, 2010, **6**, 47.
22. These studies are summarized in C. A. Jones and A. C. McCreary, *Neuropharmacol.*, 2008, **55**, 1056.



23. C. A. Winstanley, Y. Chudasama, J. W. Dalley, D. E. Theobald, J. C. Glennon and T. W. Robbins, *Psychopharmacology*, 2003, **167**, 304.
24. Z. Li, J. Ichikawa, J. Dai and H. Y. Meltzer, *Eur. J. Pharmacol.*, 2004, **493**, 75.
25. L. Bardin, A. Newman-Tancredi and R. Depoortère, *Eur. Neuropsychopharmacol.*, 2006, **16**, S431.
26. See H. Y. Meltzer and T. Sumiyoshi, *Behav. Brain Res.*, 2008, **195**, 98 for a recent review of the evidence.
27. T. Sumiyoshi, V. Bubenikova-Valesova, J. Horacek and B. Bert, *Adv. Ther.*, 2008, **25**, 1037.
28. D. A. Clemett, T. Puhani, M. S. Duxon, T. P. Blackburn and K. C. Fone, *Neuropharmacology*, 2000, **39**, 123.
29. V. Di Matteo, G. Di Giovanni, M. Di Mascio and E. Esposito, *Neuropharmacology*, 1998, **37**, 265.
30. Summarized in K. L. Marquis, A. L. Saab, S. F. Logue, J. A. Brennan, M. J. Piesla, T. A. Comery, S. M. Grauer, C. R. Ashby, Jr, H. Q. Nguyen, L. A. Dawson, J. E. Barrett, G. Stack, H. Y. Meltzer, B. L. Harrison and S. Rosensweig-Lipson, *J. Pharmacol. Exp. Ther.*, 2007, **320**, 486.
31. L. M. Redman and E. Ravussin, *Drugs Today*, 2010, **46**, 901.
32. See summary of studies at [www.clinicaltrials.gov](http://www.clinicaltrials.gov).
33. J. A. Suiciak, D. S. Chapin, S. A. McCarthy, V. Guanowsky, J. Brown, P. Chiang, R. Marala, T. Patterson, P. A. Seymour, A. Swick and P. A. Iredale, *Neuropharmacology*, 2007, **52**, 279.
34. B. L. Roth, *New Eng. J. Med.*, 2007, **356**, 6.
35. J. Dunlop, S. W. Watts, J. E. Barrett, J. Coupet, B. Harrison, H. Mazandarani, S. Nawoschik, M. N. Pangalos, S. Ramamoorthy, L. Schecter, D. Smith, G. Stack, J. Zhang, G. Zhang and S. Rosenzweig-Lipson, *J. Pharmacol. Exp. Ther.*, 2011, **337**, 673.
36. S. J. Cho, N. H. Jensen, T. Kurome, S. Kadari, M. L. Manzano, J. E. Malberg, B. Caldarone, B. L. Roth and A. P. Kozikowski, *J. Med. Chem.*, 2009, **52**, 1885.
37. A. P. Kozikowski, S. J. Cho, N. H. Jensen, J. A. Allen, A. M. Svennebring and B. L. Roth, *ChemMedChem*, 2010, **5**, 1221.
38. M. A. Geyer, *Pharmacopsychiatry*, 1998, **31**(2), 73.
39. G. Lucas and U. Spampinato, *J. Neurochem.*, 2000, **74**, 693.
40. G. K. Aghajanian and G. J. Marek, *Brain Res. Rev.*, 2000, **31**, 302.
41. A. M. Barr, V. Lehmann-Masten, M. Paulus, R. R. Gainetdinov, M. G. Caron and M. A. Geyer, *Neuropsychopharmacology*, 2004, **29**, 221.
42. A. Gozzi, V. Crestan, G. Turrini, M. Clemens and A. Bifone, *Psychopharmacology*, 2010, **209**, 37.
43. A. V. Terry Jr, J. J. Buccafusco and G. D. Bartoszyk, *Psychopharmacology*, 2005, **179**, 725.
44. L. R. Gardell, K. E. Vanover, L. Pounds, R. W. Johnson, R. Barido, G. T. Anderson, I. Veinbergs, A. Dyssegaard, P. Brunmark, A. Tabatabaei, R. E. Davis, M. R. Brann, U. Hacksell and D. W. Bonhaus, *J. Pharmacol. Exp. Ther.*, 2007, **322**, 862.

45. P. Sokoloff, B. Giros, M. P. Martres, L. Marie and J. C. Schwartz, *Nature*, 1990, **347**, 146.
46. P. Sokoloff, J. Dias, B. Le Foll, O. Guillin, L. Lerich, E. Bezard and C. Gueremy, *CNS Neurol. Disord. Drug Targets*, 2006, **5**, 25.
47. F. Micheli and C. Heidbreder, *Exp. Opin. Ther. Patents*, 2008, **18**, 821.
48. F. Micheli, *ChemMedChem*, 2011, **6**, 1152.
49. G. Stemp, T. Ashmeade, C. L. Branch, M. S. Hadley, A. J. Hunter, C. N. Johnson, D. J. Nash, K. M. Thewlis, A. K. Vong, N. E. Austin, P. Jeffrey, K. Y. Avenell, I. Boyfield, J. J. Hagan, D. N. Middlemiss, C. Reavill, G. J. Riley, C. Routledge and M. Wood, *J. Med. Chem.*, 2000, **43**, 1878.
50. H. Geneste, W. Amberg, G. Backfisch, A. Beyerbach, W. M. Braeje, J. Delzer, A. Haupt, C. W. Hutchins, L. L. King, D. R. Sauer, L. Unger and W. Wernet, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1934.
51. L. Redden, B. Rendenbach-Mueller, W. M. Abi-Saab, D. A. Katz, A. Goenjian, W. Z. Robieson, Y. Wang, S. L. Goss, N. Greco and M. D. Saltarelli, *J. Clin. Psychopharmacol.*, 2011, **31**, 221.
52. A. Graff-Guerrero, L. Redden, W. Abi-Saab, D. A. Katz, S. Houle, P. Barsoum, A. Bhathena, R. Palaparthi, M. D. Saltarelli and S. Kapur, *Int. J. Neuropsychopharmacol.*, 2010, **13**, 273.
53. M. J. Millan, C. Mannoury la Cour, F. Novi, R. Maggio, V. Audinot, A. Newman-Tancredi, D. Cussac, V. Pasteau, J.-A. Boutin, T. Dubuffet and G. Lavielle, *J. Pharmacol. Exp. Ther.*, 2008, **324**, 587.
54. M. J. Millan, J. J. Buccafusco, F. Loiseau, D. J. G. Watson, E. Decamp, K. C. F. Foster, N. Thomasson-Perret, M. Hill, E. Mocaer and J. S. Schneider, *Int. J. Neuropsychopharmacol.*, 2010, **13**, 1035.
55. F. Micheli, L. Arista, G. Bonanomi, F. E. Blaney, S. Braggio, A. M. Capelli, A. Checcia, F. Damiani, R. Di-Fabio, S. Fontana, G. Gentile, C. Griffante, D. Hamprecht, C. Marchioro, M. Mugnaini, J. Piner, E. Ratti, G. Tedesco, L. Tarsi, S. Terreni, A. Worby, C. R. Ashby Jr and C. Heidbreder, *J. Med. Chem.*, 2010, **53**, 374.
56. F. Micheli, L. Arista, B. Bertrani, S. Braggio, A. M. Capelli, S. Cremonesi, R. Di-Fabio, G. Gelardi, G. Gentile, C. Marchioro, A. Pasquarello, S. Provera, G. Tedesco, L. Tarsi, S. Terreni, A. Worby and C. Heidbreder, *J. Med. Chem.*, 2010, **53**, 7129.
57. E. Y. T. Chien, W. Liu, Q. Zhao, V. Katrich, G. W. Han, M. A. Hanson, L. Shi, A. H. Newman, J. A. Javitch, V. Cherezov and R. C. Stevens, *Science*, 2010, **330**, 1091.
58. A. H. Newman, P. Grundt, G. Cyriac, J. R. Deschamps, M. Taylor, R. Kumar, D. Ho and R. R. Luedtke, *J. Med. Chem.*, 2009, **52**, 2559.
59. L. J. Bristow, M. S. Kramer, J. Kulagowski, S. Patel, C. I. Ragan and G. R. Seabrook, *Trends Pharm. Sci.*, 1997, **18**, 186.
60. S. Linz, J. Müller, H. Hübner, P. Gmeiner and R. Troschütz, *Bioorg. Med. Chem.*, 2009, **17**, 4448.
61. P. Hertel, M. Didriksen, B. Pouzet, L. T. Brennum, K. K. Søby, A. K. Larsen, C. T. Christoffersen, T. Ramirez, M. M. Marcus, T. H. Svensson,

- V. Di Matteo, E. Esposito, B. Bang-Andersen and J. Arnt, *Eur. J. Pharmacol.*, 2007, **573**, 148.
62. L. Bardin, A. Auclair, M. S. Kleven, E. P. M. Prinssen, W. Koek, A. Newman-Tancredi and R. Depoortère, *Behav. Pharmacol.*, 2007, **18**, 103.
63. A. C. McCreary, J. C. Glennon, C. R. Ashby, Jr, H. Y. Meltzer, Z. Li, J.-H. Reinders, M. B. Hesselink, S. K. Long, A. H. Herremans, H. van Stuivenberg, R. W. Feenstra and C. G. Kruse, *Neuropsychopharmacology*, 2007, **32**, 78.
64. D. Boulay, R. Depoortère, C. Louis, M. Lacave, M. T. Lucas and G. Griebel, *Pharmacol. Biochem. Behav.*, 2011, **97**, 428.
65. T. Ishibashi, T. Horisawa, K. Tokuda, T. Ishiyama, M. Ogasa, R. Tagashira, K. Matsumoto, H. Nishikawa, Y. Ueda, S. Toma, H. Oki, N. Tanno, I. Saji, A. Ito, Y. Ohno and M. Nakamura., *J. Pharmacol. Exp. Ther.*, 2010, **334**, 171.
66. H. Silver, *Int. Clin. Psychopharmacol.*, 2003, **18**, 305.
67. D. Mazeh, B. Shahal, R. Saraf and Y. Melamed, *J. Clin. Pharmacol.*, 2004, **24**, 653.
68. P. Smid, H. K. A. C. Coolen, H. G. Keizer, R. van Hess, J.-P. de Moes, A. P. den Hartog, B. Stork, R. H. Plekkenpol, L. C. Niemann, C. N. Stroomer, M. T. Tulp, H. H. van Stuivenberg, A. C. McCreary, M. B. Hesselink, A. H. Herremans and C. G. Kruse, *J. Med. Chem.*, 2005, **48**, 6855.
69. J. A. Brennan, R. Graf, S. M. Grauer, R. L. Navarra, C. M. Pulicicchio, Z. A. Hughes, Q. Lin, C. Wantuch, S. Rosenzweig-Lipson, F. Pruthi, M. Lai, D. Smith, W. Goutier, M. van de Neut, A. J. Robichaud, D. Rotella, R. W. Feenstra, C. Kruse, P. Broqua, C. E. Beyer, A. C. McCreary, M. H. Pausch and K. L. Marquis, *J. Pharmacol. Exp. Ther.*, 2010, **332**, 190.
70. D. P. Rotella, G. R. McFarlane, A. Greenfield, C. Grosanu, A. J. Robichaud, R. A. Denny, R. W. Feenstra, S. Núñez-García, J. H. Reinders, M. Neut, A. McCreary, C. G. Kruse, K. Sullivan, F. Pruthi, M. Lai, J. Zhang, D. M. Kowal, T. Carrick, S. M. Grauer, R. L. Navarra, R. Graf, J. Brennan, K. L. Marquis and M. H. Pausch, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5552.
71. Y. Yan, P. Zhou, D. P. Rotella, R. Feenstra, C. G. Kruse, J.-H. Reinders, M. van der Neut, M. Lai, J. Zhang, D. M. Kowal, T. Carrick, K. L. Marquis, M. H. Pausch and A. J. Robichaud, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2983.
72. B. L. Roth, D. J. Sheffler and W. K. Kroeze, *Nat. Rev. Drug Discovery*, 2004, **3**, 353.

## CHAPTER 4

# *Glutamatergic Approaches for the Treatment of Schizophrenia*

JOHN A. MORROW,<sup>\*a</sup> ROBERT GILFILLAN<sup>b</sup> AND  
STUART A. NEALE<sup>c</sup>

<sup>a</sup> Neuroscience and Ophthalmology, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, USA; <sup>b</sup> Discovery Chemistry, Merck Research Laboratories, 770 Sumneytown Pike, West Point, Pennsylvania 19486, USA; <sup>c</sup> Neurexpt Ltd, Ground Floor, 2 Woodberry Grove, North Finchley, London, N12 0DR, UK

\*Email: john.morrow@merck.com

## 4.1 Introduction

Schizophrenia is a complex and serious neurodevelopmental psychiatric disorder that affects approximately 1% of the world population and is one of the leading causes of chronic disability. It is the most common severe mental illness, and typically emerges in late adolescence or early adulthood. Prognosis is generally poor with sufferers generally experiencing a life-long pattern of ongoing disability with relapses of acute psychotic episodes. Symptoms of schizophrenia are typically divided into three main classes, which may be present to different degrees in different patients. Positive symptoms (*e.g.* delusions, hallucinations, disorganized speech and behaviour), negative symptoms (*e.g.* social withdrawal, avolition, blunted affect) and cognitive deficits (*e.g.* impaired sustained attention, executive function and working memory).<sup>1</sup> Although prominence is often given to positive symptoms, which appear more dramatic and often characterize relapses, disability results

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

particularly from negative symptoms and cognitive deficits. These features are more chronic and inflexible and have a greater impact on long-term prognosis.<sup>2,3</sup>

The underlying aetiology of schizophrenia is very poorly understood. It is a highly heterogeneous disorder with no common identifiable pathology, although a number of environmental and genetic contributions have been proposed. The dopamine hypothesis of schizophrenia attributes positive symptoms to hyperactivity of the mesolimbic dopaminergic pathway, whereas negative and cognitive symptoms are associated with hypoactivity of the mesocortical dopaminergic pathway.<sup>4-9</sup> It is supported by observations that all established marketed antipsychotic drugs are dopamine D<sub>2</sub> receptor antagonists and agents such as amphetamine and cocaine that raise dopamine levels in the brain cause symptoms resembling those present in psychoses. This is also strongly supported by positron emission tomography (PET) imaging studies in schizophrenics demonstrating hyper-responsive striatal dopamine pathways.<sup>5-9</sup>

While newer second-generation “atypical” antipsychotic drugs (SGAs) have superior side-effect profiles and tolerability (*e.g.* rarely causing extra-pyramidal side-effects (EPS), reduced prolactin secretion) in comparison with older first-generation “typical” antipsychotics (FGAs), these treatments only have proven efficacy for the treatment of positive symptoms of schizophrenia.<sup>10</sup> Indeed, there is still relatively little evidence to suggest that SGAs provide a better functional outcome for patients than FGAs,<sup>11</sup> an observation highlighted by large-scale clinical studies such as CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness<sup>12</sup>) conducted in the USA and CUtLASS (Cost Utility of the Latest Antipsychotic Drugs in Severe Schizophrenia<sup>13,14</sup>) in the UK.

There are significant unmet needs in treating schizophrenia, from both efficacy and side-effect perspectives. Despite appropriate treatment with antipsychotic drugs, many schizophrenics fail to respond to either FGAs or SGAs. Some very significant side-effects associated with most SGAs are weight gain, glucose dysregulation and dyslipidemia.<sup>15,16</sup> Major unmet medical needs include the effective treatment of both negative symptoms<sup>17</sup> and cognitive dysfunction and accordingly these are the primary focus for new therapeutic approaches.<sup>18,19</sup>

The glutamate hypothesis of schizophrenia was originally based on the observation that non-competitive NMDA receptor antagonists such as ketamine and PCP induce schizophrenia-like symptoms in normal individuals and exacerbate positive, negative and cognitive symptoms in patients with schizophrenia.<sup>20-23</sup> Indeed, the symptoms observed across positive, negative and cognitive domains during acute ketamine challenge in normal individuals exhibit a similar pattern to those observed in schizophrenia. Further support for the glutamate hypothesis comes from biochemical evidence providing the first direct evidence of potential NMDA receptor hypofunction.<sup>24</sup> Specifically, NRG1-mediated suppression of NMDA receptor function, possibly *via* enhanced activation of ErbB4, was enhanced in *post mortem* prefrontal cortex from schizophrenic subjects relative to healthy controls. Overall, this evidence

has led to the evolution of a number of novel therapeutic strategies for the treatment of schizophrenia based around the enhancement of glutamatergic transmission.

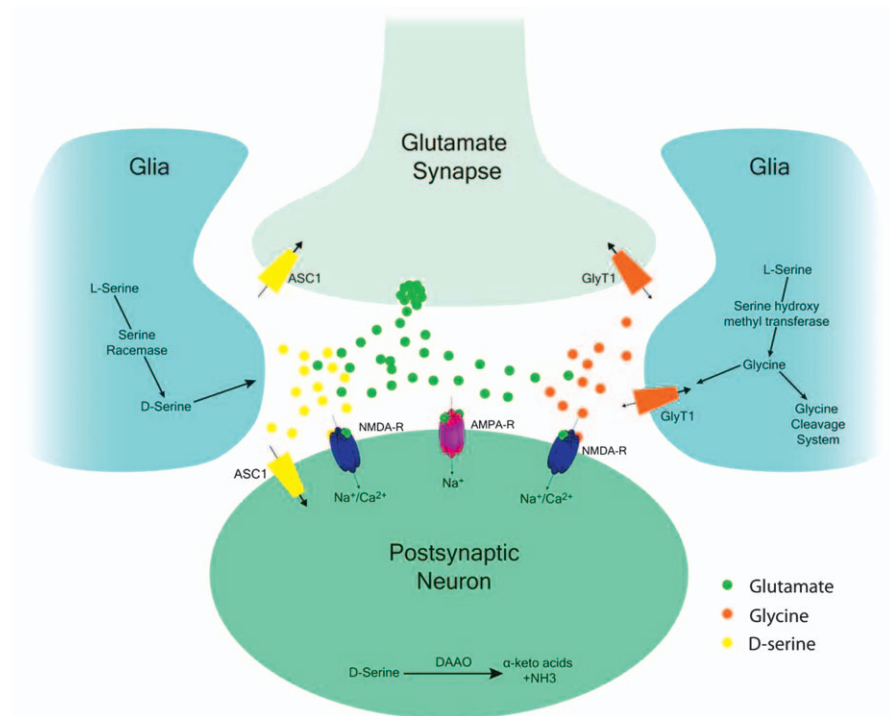
This chapter focuses on the key therapeutic strategies for augmentation of glutamatergic neurotransmission in schizophrenia currently under investigation by the pharmaceutical industry. We will discuss the advances that have been made in our understanding of how these strategies augment glutamatergic neurotransmission and focus on the preclinical and, indeed, emerging clinical evidence that suggest that a number of these approaches may serve as a promising new therapy for the treatment of schizophrenia.

## 4.2 Targeting the Co-agonist Site of the NMDA Receptor

The most direct approaches towards addressing the hypoglutamatergic deficit in schizophrenia are those targeting the co-agonist site of the NMDA receptor by raising the concentration of either glycine or D-serine (Figure 4.1). This is also considered to have the advantage of avoiding the potential excitotoxic consequences associated with direct activation *via* the glutamate site. Some encouragement for this approach has been provided by a number of small clinical studies involving administration of either of the full agonists glycine<sup>25,26</sup> or D-serine,<sup>27</sup> the partial agonist D-cycloserine<sup>28–30</sup> or the naturally occurring low-affinity GlyT1 inhibitor and substrate sarcosine<sup>31,32</sup> as add-on therapy to FGAs or SGAs. The most prominent affect observed was a significant improvement in negative symptoms,<sup>33</sup> while positive and cognitive domains were also noted in some, but not all of the studies. However, a recent, more comprehensive multi-centre trial (the CONSIST study<sup>34</sup>) examining the effect in schizophrenic individuals of adjunctive glycine or D-cycloserine for negative symptoms and cognition failed to demonstrate significant improvement by either agent. All of these agents are poorly CNS penetrant and a key issue with the interpretation of these data is the lack of certainty that sufficient CNS levels and, indeed, synaptic levels of these agents have been achieved in order to effect the requisite target engagement. To overcome these deficiencies, a series of more efficient approaches for raising the synaptic levels of either glycine or D-serine are currently being investigated by a number of pharmaceutical companies.

### 4.2.1 Glycine Transporter Inhibitors

The amino acid glycine has two major roles in the regulation of mammalian central neurotransmission. In addition to its modulation of excitatory neurotransmission through its action as an obligatory co-agonist with glutamate at the NMDA receptor<sup>35</sup> (Figure 4.1), it is the major inhibitory neurotransmitter in the caudal brain and spinal cord where its action is mediated by the strychnine-sensitive glycine receptor to produce inhibitory post-synaptic potentials.<sup>36</sup>



**Figure 4.1** Mechanisms for modulation of NMDA receptor signalling by increasing levels of D-serine and glycine. NMDA receptors are activated by glutamate released from pre-synaptic neurons and a co-agonist of either glycine or D-serine. D-serine is synthesized by the enzyme serine racemase, and D-serine levels in the synapse are regulated by the transporter ASC-1 and enzymatic breakdown by DAAO. Glycine is synthesized in glia, and levels are modulated by activity of the transporter GlyT1.

The synaptic effects of glycine are terminated by rapid reuptake into the pre-synaptic terminal or surrounding glial cells *via* two distinct high-affinity glycine transporters, GlyT1 and GlyT2, both belonging to the  $\text{Na}^+/\text{Cl}^-$  dependent family of neurotransmitter transporters.<sup>37–44</sup> Both exist as multiple isoforms as a result of alternative splicing or use of different promoters. Three predominant variants of human GlyT1 (GlyT1a, b and c) have been described, each differing only in their N-terminal region.<sup>39,44,38</sup> GlyT1 exhibits widespread distribution throughout the CNS. In the caudal brain and spinal cord, GlyT1 is predominantly localized in glial cells where it is probably involved in the regulation of inhibitory glycinergic neurotransmission.<sup>45</sup> In the forebrain, however, it is localized to subpopulations of pre- and post-synaptic glutamatergic terminals in close proximity to NMDA receptors.<sup>46</sup> By contrast, GlyT2 is present on inhibitory glycinergic neurons in caudal brain and spinal cord implying a role in the regulation of glycinergic neuronal activity.



The physiological role of these transporters has largely been defined through the study of genetically modified animal models. Both GlyT1<sup>-/-</sup> and GlyT2<sup>-/-</sup> animals appear normal at birth but die within one day and two weeks, respectively.<sup>47,48</sup> GlyT2<sup>-/-</sup> animals display motor deficiencies such as tremor, muscle spasticity and poor motor coordination as a result of glycine depletion at the glycinergic synapses. GlyT2 therefore plays a crucial role in recycling synaptically released glycine back into the cytoplasmic compartment for loading into synaptic vesicles for subsequent release upon neuronal stimulation. GlyT1<sup>-/-</sup> animals on the other hand exhibit poor motor responses and marked respiratory deficiency. This appears to be a result of excessive stimulation of inhibitory glycine receptors as respiratory activity in brain stem slices from GlyT1<sup>-/-</sup> neonates can be normalized by application of strychnine,<sup>47</sup> indicating a major role for GlyT1 in the regulation of glycine levels at inhibitory glycinergic receptors.

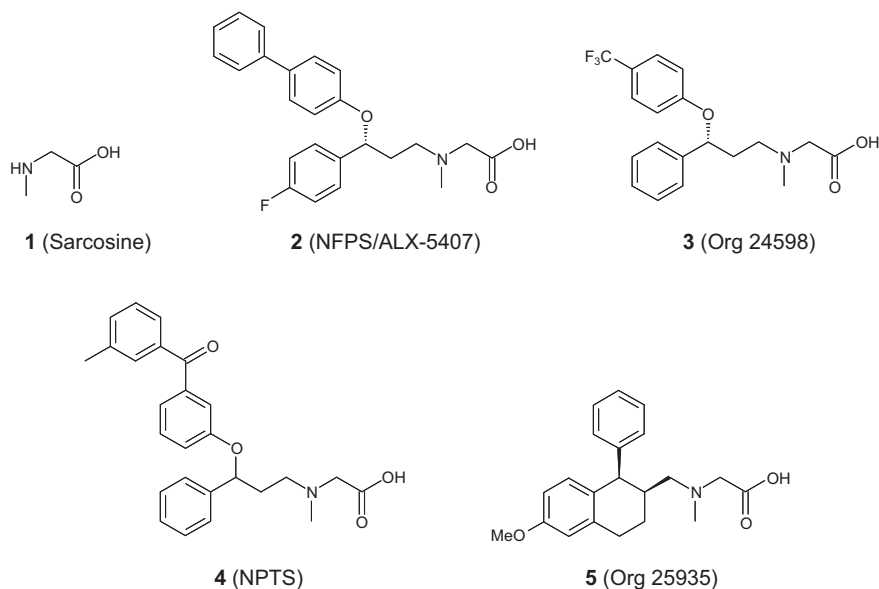
Evidence that GlyT1 is able to regulate glycine levels at the NMDA receptor comes from studies using heterozygous<sup>49</sup> and forebrain selective homozygous knockout mice.<sup>50</sup> Both of these lines exhibited enhanced NMDA-dependent synaptic responses in acute hippocampal slices. Furthermore, the heterozygous mice exhibited enhanced spatial memory in the water maze compared to wild-type litter mates together with protection against disruptions in sensory gating.<sup>49</sup> These findings not only highlight the role that GlyT1 plays in the regulation of glutamatergic neurotransmission, but also suggest that inhibition of GlyT1 may provide antipsychotic and cognitive enhancement in the treatment of schizophrenia.

A large body of research on GlyT1 inhibitors has emerged in recent years and has produced a large number of GlyT1 inhibitors exhibiting wide structural diversity (see<sup>51-53</sup>). These compounds are generally classified as either amino acid based *e.g.* NFPS, Org 24598 and Org 25935, or non-amino acid based compounds *e.g.* SSR504734, SSR103800, RG1678 and PF-3463275. Several examples of these have been demonstrated to elevate extra-cellular glycine levels in brain structures and spinal cord<sup>54-59</sup> and enhance NMDA receptor activity.<sup>56-58</sup> This mechanistic proof-of-principle has provided the platform for exploring the therapeutic utility of these molecules in the treatment of schizophrenia.

#### 4.2.1.1 Amino Acid Based GlyT1 Inhibitors

The first selective GlyT1 inhibitors to be described were based on the naturally occurring low potency (IC<sub>50</sub> = 37 μM) GlyT1 inhibitor and substrate sarcosine (Figure 4.2).<sup>39</sup> The best characterized examples include NFPS ((*R*)-*N*-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy) propyl] sarcosine; also known as ALX 5407) and Org 24598 (the *R*-(-) isomer of Org 24461). NFPS is a potent GlyT1 inhibitor (IC<sub>50</sub> = 3–36 nM) with no appreciable activity at GlyT2 and, in contrast to sarcosine, is not a transporter substrate.<sup>60-62</sup> Org 24598 is also a potent (IC<sub>50</sub> = 7–126 nM) GlyT1 inhibitor with no appreciable off-target activities.<sup>63,60</sup> The discovery of these compounds led to the subsequent identification of a number of amino acid based GlyT1 inhibitors, some examples of





**Figure 4.2** Sarcosine and amino acid based GlyT1 inhibitors.<sup>305,304,306</sup>

which are illustrated in Figure 4.2. It is notable that the known amino acid inhibitors share a common pharmacophore consisting of the amino acid, typically glycinergic fragment, and two aromatic groups some four to five bond lengths distal from the basic nitrogen. The most advanced compound from this class, Org 25935, has progressed to phase II clinical studies for the treatment of schizophrenia.

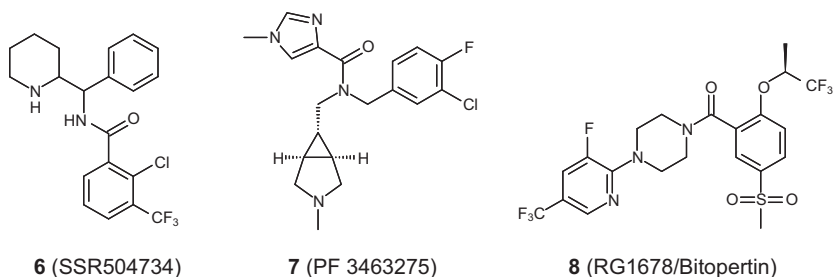
NFPS and Org 24598 have been subject to a battery of tests typically used to characterize antipsychotic efficacy of typical and atypical antipsychotic drugs (see Chapter 20, Animal Models of Psychiatric Disorders, Mark Tricklebank). Both NFPS and Org 24461 were able to reverse PCP-induced hypolocomotion and changes in EEG power spectra in rats as might be expected from their mechanism of action. However, only Org 24461 was able to reverse amphetamine-induced hyperlocomotion in rats.<sup>64</sup> Neither compound had any effect on apomorphine-induced climbing and stereotypic behaviour in mice, nor did they induce catalepsy (typically used to assess side-effect potential) in rats.<sup>64</sup> In studies to investigate the effect of these compounds on attentional processes, NFPS was demonstrated to reverse MK-801-induced deficits in prepulse inhibition (PPI)<sup>65</sup> as well as ameliorating the innate PPI deficit in the DBA mouse strain.<sup>66</sup> This compound also reduced baseline PPI in normal animals,<sup>65</sup> which may indicate a potential adverse effect of GlyT1 inhibition under non-challenged conditions. Org 24598 was also able to ameliorate the PPI deficit in DBA mice as well as partially reversing PPI deficiency induced by neonatal lesion in ventral hippocampus.<sup>67</sup> NFPS also reverses persistent latent inhibition (LI) induced by MK-801.<sup>65</sup>

The effects of GlyT1 inhibitors on cognition have been investigated in a number of learning and memory paradigms. NFPS in particular has been shown to reverse MK-801-induced deficits in spatial reference memory<sup>68</sup> and object recognition memory.<sup>69,70</sup> These tests were all performed following acute treatment with MK-801 and NFPS; however, in one particularly interesting study, sub-chronic NFPS treatment was also able to reverse a long-term deficit in recognition memory in mice induced by repeated (10-day) treatment of PCP.<sup>71</sup> Taken together, these findings suggest that amino acid based GlyT1 inhibitors may serve as potential treatments for positive and cognitive symptoms of schizophrenia.

#### 4.2.1.2 Non-amino Acid Based GlyT1 Inhibitors

The prototypical non-sarcosine based GlyT1 inhibitors, and best studied examples to date, are the Sanofi compounds SSR504734<sup>72</sup> (Figure 4.3) and SSR103800 (structure not disclosed). Both are potent and selective inhibitors of GlyT1 ( $IC_{50}$  = 18 nM and 1.9 nM, respectively, at human GlyT1).<sup>56,57</sup> Other notable examples of potent, selective non-amino acid GlyT1 inhibitors include PF3453276<sup>73,74</sup> (Pfizer) and RG1678<sup>75,76</sup> (Roche) (Figure 4.3). The presence of an aromatic amide appears to be a common, but not essential, feature amongst many reported non-amino acid GlyT1 inhibitors. However, other structural features differ considerably and, unlike the amino-acid derived GlyT1 inhibitors, do not share a common pharmacophore.

Both SSR504734 and SSR103800 have been demonstrated to reverse MK-801-induced hyperlocomotion at doses that were without effect on spontaneous locomotor activity.<sup>56,57,77</sup> However, studies to determine the effect of these compounds on amphetamine-induced stereotypic behaviour have produced mixed results. Contrary to what is typically observed with classical dopamine antagonist antipsychotic drugs, SSR504734 exacerbated amphetamine induced hyperlocomotion,<sup>78</sup> while SSR103800 failed to reverse the locomotor effect of amphetamine.<sup>56</sup> SSR504734 was able to ameliorate the motor sensitivity to amphetamine induced by neonatal PCP treatment.<sup>57</sup> The reasons underlying these differences are not clear but may be due to subtle differences in the way these compounds impact the dopamine system.<sup>79</sup> SSR504734 has been



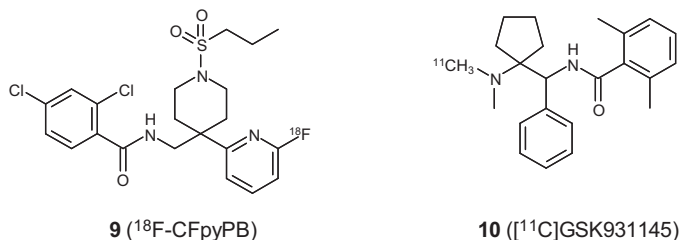
**Figure 4.3** Non-amino acid based GlyT1 inhibitors.

demonstrated to increase dopamine levels in the prefrontal cortex in rats<sup>57</sup> and facilitate glutamate-dependent dopamine release in the nucleus accumbens while having no effect on basal dopamine levels.<sup>80</sup> A systematic comparison of these compounds in such tests will be required to understand their different impact on amphetamine induced behaviour. Stronger support for the anti-psychotic potential of these compounds comes from studies on attentional processes with both SSR 504734 and SSR103800 able to ameliorate the innate PPI deficiency in DBA mice<sup>56,57</sup> and reverse MK-801-induced persistent latent inhibition (LI).<sup>81</sup> SSR103800 was additionally demonstrated to counter amphetamine-induced disruption of LI while in a neurodevelopmental model, where adult animals display abnormally persistent LI having been neonatally treated with a nitric oxide synthase inhibitor, SSR504734 reverted LI back to control levels.<sup>81</sup> Both compounds have also been demonstrated to reverse cognitive deficits in social recognition memory induced by neonatal PCP administration,<sup>56,57,82</sup> thereby suggesting their potential use for the treatment of cognitive deficits and perhaps even negative symptoms associated with schizophrenia.

Another interesting non-amino acid based GlyT1 inhibitor, recently developed by Pfizer, is PF-3463275.<sup>74</sup> While this compound had no effect on ketamine-induced positive symptom (hallucinatory)-like behaviour, it was demonstrated to ameliorate spatial working memory deficits induced by ketamine in Rhesus monkeys,<sup>83</sup> again suggesting potential efficacy for cognitive deficits associated with schizophrenia. PF-3463275 was reported to have entered phase II clinical development as an adjunctive therapy to ongoing atypical antipsychotic treatment for evaluation for the treatment of cognitive deficits in subjects with chronic schizophrenia; however, clinical development was halted for reasons as yet undisclosed ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)).

Perhaps the most significant advance in the GlyT1 field to date has emerged from Roche with their selective and potent GlyT1 inhibitor RG1678 (Bitopertin).<sup>76</sup>

The limited preclinical data published with this compound to date has demonstrated that it is able to attenuate hyperlocomotion in mice after administration with the NMDA glycine site antagonist L-687,414 and produce a robust and sustained increase of extra-cellular glycine levels in rat striatum following oral administration.<sup>76</sup> Results from a double-blind, placebo-controlled, multi-centre phase IIb clinical trial in patients ( $n = 323$ ) with schizophrenia were reported by Roche for RG1678 in November 2009. The compound was administered as an adjunctive therapy at three doses, 10, 30 and 60 mg, to patients stabilized on antipsychotic therapy. The two lower doses (but not the high-dose group) demonstrated significant improvements in negative symptoms as judged by the PANSS rating scale with differences in overall physician's assessment (clinical global impression; CGI) significantly different just for the 10 mg group. The 10 mg group also displayed a trend toward functional improvement as judged by the personal and social performance (PSP) rating scale ([www.Roche.com](http://www.Roche.com)). While the observed effect sizes were small, they were enough to persuade Roche to commence phase III studies with



**Figure 4.4** GlyT1 PET ligands.

RG1678, where it is now under investigation for negative symptoms and sub-optimally controlled patients.

Roche also reported data from studies utilizing a novel GlyT1 PET ligand, which suggested that the optimal target occupancy for therapeutic effect is around 50%. It is clear that PET ligand such as the one used by Roche together with those published by Merck ( $[^{18}\text{F}]$ CFPyPB<sup>84</sup>) and GSK ( $[^{11}\text{C}]$ GSK931145<sup>85</sup>) (Figure 4.4) will prove to be valuable target engagement biomarkers to define precisely human dose prediction required for optimal therapeutic effect.

#### 4.2.1.3 Amino Acid versus Non-amino Acid Based GlyT1 Inhibitors

While there are similarities in the pharmacological properties of the amino acid and non-amino acid based GlyT1 inhibitors, a number of important differences have emerged in recent years. Amino acid based compounds are widely perceived as suffering from a wide range of side-effects such as motor impairments and decreased respiratory activity, which can be fatal upon chronic dosing.<sup>66,86</sup> These effects are thought to be mediated by activation of strychnine-sensitive glycine receptors in the cerebellum and brainstem and, indeed, some of these side-effects can be blocked by strychnine.<sup>66,86</sup> This has been proposed to be a consequence of the irreversible and non-competitive mode of action that has been ascribed for some amino acid based inhibitors, whereas non-amino acid inhibitors with their surmountable and competitive mode of action are considered to possess a higher degree of safety margin.<sup>87</sup> However, there are notable exceptions to this rule since not all amino acid based compounds are irreversible inhibitors (e.g. Org 25935 has a fully reversible mode of action; unpublished observations; J. Morrow, A. Porter, F. Thomson and R. Gilfillan) and the non-amino acid inhibitor RG1678 has a non-competitive mode of action<sup>66</sup> (referred to as Roche 7 in this publication). Despite its non-competitive mode of action, RG1678 did not induce motor side-effects (also described as obstinate progression).<sup>66</sup> This was proposed to be a result of reduced residence time at GlyT1, whereas compounds with more prolonged residence times were more prone to inducing side-effects.

### 4.2.2 D-serine: a Selective NMDA Receptor Modulator

Although the role of NMDA receptor co-agonist was originally ascribed to glycine,<sup>35</sup> there is evidence that the endogenous co-agonist at many central synapses is the amino acid D-serine<sup>88,89</sup> (Figure 4.1). D-serine is inactive at the strychnine-sensitive glycine receptor,<sup>90</sup> therefore modulating levels of D-serine may selectively enhance NMDA receptor activity for the treatment of schizophrenia<sup>91</sup> whilst avoiding side-effects of GlyT1 inhibition, which have been attributed to glycine activating strychnine-sensitive glycine receptors.<sup>66,86</sup>

D-serine activates heterologously expressed NMDA receptors at sub-micromolar concentrations and is at least as potent as glycine.<sup>92,93</sup> In native receptor preparations, however, D-serine has a greater effect on NMDA receptor activation than glycine, which may in part be due to glycine transporters preventing glycine accessing the NMDA receptor.<sup>94,95</sup> D-serine is predominately localized to astrocytes<sup>96</sup> (although there is evidence of D-serine synthesis in neurons<sup>97</sup>), where it is synthesized from L-serine by the enzyme serine racemase<sup>98</sup> (Figure 4.1). In the brain, D-serine levels mirror expression of NMDA receptors<sup>99,100</sup> and it is released in a calcium-dependent fashion from glial cells in response to activation of glutamate receptors.<sup>101</sup> Experiments where D-serine has been enzymatically degraded, whereby loss of D-serine results in attenuation of NMDA receptor-mediated events,<sup>102–104</sup> provide key evidence that D-serine is the endogenous co-agonist.

A body of evidence suggests increasing levels of D-serine may be of benefit in the treatment of schizophrenia, an effect hypothesized to be *via* modulation of NMDA receptor function. Importantly, *in vivo* data suggest the NMDA receptor co-agonist site is not saturated<sup>105,106</sup> and may therefore be available as a target for drug treatment. Preclinical studies demonstrate enhanced NMDA receptor-dependent processes in response to increased levels of D-serine<sup>104,107</sup> and amelioration of symptoms in behavioural models of schizophrenia.<sup>108,109</sup> In schizophrenic patients, the levels of D-serine in cerebrospinal fluid was found to be significantly decreased<sup>110</sup> and, consistent with this observation, studies of genes associated with susceptibility to psychiatric disorders have identified a link with those relevant to regulation of D-serine levels with the risk of schizophrenia<sup>111–113</sup> (<http://www.szgene.org>) and with bipolar disorder.<sup>114</sup> Meta-analysis of a number of clinical studies suggests D-serine treatment may be efficacious in multiple symptom domains.<sup>91,115</sup>

#### 4.2.2.1 Sodium Independent Alanine–Serine–Cysteine Transporter 1 (ASC-1)

A number of transporters have been identified for D-serine, but the transporter ASC-1 appears to be the main uptake mechanism for D-serine in the mammalian CNS<sup>116</sup> (Figure 4.1). ASC-1 is coded by SLC7A10 (solute carrier family 7 (cationic amino acid transporter, y<sup>+</sup> system) member) with the human ASC-1 gene mapping to human chromosome 19, region q12–q13.<sup>117,118</sup> There are relatively few data linking ASC-1 modulation to schizophrenia; however,

several factors point towards it being a potential target for development of new therapeutic agents that modulate NMDA receptor signalling. ASC-1 has a widespread distribution in the mammalian CNS where it is located in pre-synaptic terminals and dendrites with a general expression pattern somewhat mirroring expression of NMDA receptors.<sup>119,120</sup> Furthermore, mutant mice lacking ASC-1 developed tremors and seizures that were ameliorated with NMDA receptor antagonists.<sup>117</sup> To date there are relatively few published studies directly linking ASC-1 with schizophrenia; however, ASC-1 protein levels are reported to be altered in schizophrenia although, perhaps unexpectedly, ASC-1 levels are down-regulated, an observation suggested to be concomitant with down-regulation of serine levels.<sup>121</sup>

Our understanding of the role and function of ASC-1 and its potential as a target for the treatment of schizophrenia or other psychiatric disorders will be greatly advanced by the discovery of appropriate pharmacological tools. It has been reported that *S*-methyl-cysteine is a weak ( $K_i = 62 \mu\text{M}$ ) inhibitor of [ $^3\text{H}$ ]D-serine transport in an assay using HEK293 cells expressing human ASC-1.<sup>122</sup> However, the weak potency and non-drug-like characteristics of this compound limit its use and at the time of writing no further reports of ASC-1 inhibitors were found in the scientific literature or patents.

#### 4.2.2.2 D-Amino Acid Oxidase (DAAO)

There is a body of literature describing the role of DAAO and the potential value of the enzyme as a target for the treatment of schizophrenia and this has been the subject of recent extensive reviews.<sup>123–125</sup>

DAAO is a FAD-containing flavoenzyme discovered nearly 80 years ago.<sup>126</sup> This enzyme catalyzes the oxidative deamination of D-amino acids to  $\alpha$ -keto acids and ammonia<sup>127</sup> (Figure 4.1). Although DAAO expression in the brain has been known for over 40 years,<sup>128</sup> a physiological role in the mammalian CNS was unclear until relatively recently when D-amino acids were identified in the CNS.<sup>124,128</sup>

The human DAAO gene is located at chromosome 12q24 and encodes a protein consisting of 347 amino acids.<sup>129</sup> In humans, DAAO is detected in the brain and in some peripheral tissues such as liver and kidney. Assays of enzyme activity detect strong activity in the hindbrain, but little activity of DAAO in the forebrain. Despite the apparent lack of forebrain activity, DAAO is, however, detected in forebrain neurones<sup>130</sup> and there are preclinical and clinical data that suggest disruption of DAAO activity may affect brain functions associated with frontal regions and may be of benefit in the treatment of schizophrenia.

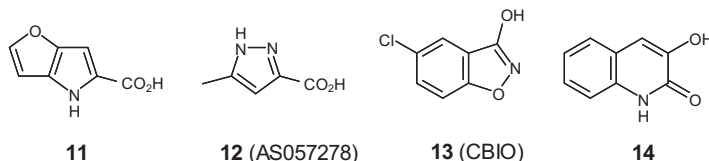
Although there are conflicting findings, a number of genetic association studies point to a link between DAAO and schizophrenia<sup>112,131,132</sup> (www.szgene.org). *Post mortem* studies of schizophrenic patients show increased activity of DAAO in the prefrontal cortex and cerebellum,<sup>133,134</sup> and functional studies in humans link genetic variation in DAAO with altered

neuronal activity and cognitive functions of relevance to schizophrenia such as sensorimotor gating and working memory.<sup>131,132,135</sup>

Several, although by no means all, genetic association studies have also implicated DAAO in schizophrenia through linkage to a region of chromosome 13q32, which harbours two genes, G72 and G30, posited to code for proteins involved in functional regulation of DAAO activity<sup>135–141</sup> (although the link with DAAO is somewhat controversial<sup>142–144</sup>). In mice, expression of the human G72/G30 gene locus results in behaviours that bear similarity to those seen in schizophrenia,<sup>140</sup> while in humans G72 may show increased expression in the prefrontal cortex of schizophrenic patients (G30 expression correlates with G72 expression, perhaps supportive of a role in G72 regulation).<sup>145</sup> G72 genotypes have also been associated with some deficits in cognitive performance in humans.<sup>135,146,147</sup>

A number of preclinical studies have been conducted to elucidate the role of DAAO in schizophrenia. Many studies have been performed on a mouse strain (ddY/DAO-) that lacks DAAO activity<sup>148</sup> resulting in elevated D-serine levels<sup>149</sup> and increased occupancy of the NMDA receptor co-agonist site.<sup>150–152</sup> These mice show significantly shorter platform search times in the Morris water maze, and significant increases in the magnitude of long-term potentiation recorded in the CA1 region of the hippocampus when compared to wild-type mice<sup>151</sup> and, consistent with high levels of DAAO activity in the cerebellum,<sup>124</sup> also show evidence of improved motor performance.<sup>152</sup> In other behavioural tests, ddY/DAO- mice show some lessening of PCP-induced hyperlocomotion, compared to wild-type mice.<sup>152</sup> Assessment of PPI has yielded conflicting results: one study reported no differences in PPI between wild-type and mutant mice,<sup>152</sup> although the authors suggest that this might have been better assessed in animals with NMDA receptor challenge; however, a second study<sup>153</sup> found significant differences in PPI of the mutant mice compared to controls. In these studies the disruptive effect of a challenge with NMDA receptor antagonists was also tested: the non-competitive NMDA receptor antagonist MK-801, tested at 0.3 mg kg<sup>-1</sup>, was equally effective in mutant and normal control mice, whereas there was an increased sensitivity to administration of the competitive antagonist SDZ 220-581 (5 mg kg<sup>-1</sup>) compared to normal mice; why the antagonists produced differential effects is not clear, although it may relate to the concentration tested and resulting magnitude of effect on PPI.<sup>153</sup> Mice with a mutation (Grin1D481N) known to result in NMDA receptor hypofunction *via* a decrease in affinity at the co-agonist site<sup>154</sup> demonstrate behavioural deficits comparable to those seen in schizophrenic patients;<sup>155</sup> combining this mutation with ddY/DAO- has enabled the effect of the loss of DAAO activity to be studied in a model of sustained NMDA receptor hypofunction.<sup>155</sup> In mice with the double mutation, loss of DAAO activity appeared to lessen the effects of the NMDA receptor hypofunction, with improvements in social approach and spatial memory, reversal of abnormally persistent latent inhibition and partial normalization of startle reactivity.<sup>156–157</sup> Of possible concern if DAAO inhibitors are to be used therapeutically is the fact that the ddY/DAO- mice reportedly exhibit increased levels of anxiety.<sup>158</sup>





**Figure 4.5** D-Amino acid oxidase inhibitors.

Considerable progress in the discovery of DAAO inhibitors has been made over the past few years and the area has been reviewed recently.<sup>123–125</sup> Several structural classes of inhibitor have now been described with examples of each illustrated in Figure 4.5. These include heterocyclic and heterobicyclic carboxylic acids, exemplified by compound **11**<sup>159</sup> and 6-chlorobenzo[d]isoxazol-3-ol (CBIO),<sup>160</sup> respectively, benzoxazoles such as 5-methyl-1H-pyrazole-3-carboxylic acid (AS057278)<sup>161</sup> and hydroxyquinolinones such as compound **14**.<sup>162</sup>

Studies with DAAO inhibitors (CBIO and AS057278) have revealed some differences, and conflicting data with regard to effects on measures relevant to schizophrenia. This contrasts with administration of D-serine, for which there are a number of positive data. This may perhaps be due to the fact that whilst the DAAO inhibitors increase brain D-serine levels, they do so modestly when compared to dosing D-serine itself.<sup>123</sup>

CBIO is a competitive DAAO inhibitor ( $IC_{50}$  of 188 nM).<sup>160</sup> When administered orally CBIO has no significant effect on plasma D-serine, nor does it affect PPI; however, when co-applied with D-serine ( $30 \text{ mg kg}^{-1}$ ) the levels of D-serine in the frontal cortex were increased compared to D-serine administered alone. A significant attenuation of PPI was also observed, a result not obtained by D-serine alone (although at higher doses, D-serine affected PPI when applied in the absence of CBIO).<sup>163</sup> D-serine was found to be effective at attenuating effects of MK-801 and amphetamine, whereas a single dose of a DAAO inhibitor ( $200 \text{ mg kg}^{-1}$ ; rat DAAO  $EC_{50} = 114 \text{ nM}$ ) was ineffective.<sup>123,164</sup> Although the inhibitor produced significant increases in brain D-serine levels, the dose of D-serine that was active ( $1,280 \text{ mg kg}^{-1}$ ) increased CSF D-serine levels some 40-fold higher than was achieved by the DAAO inhibitor ( $200 \text{ mg kg}^{-1}$ ). The inhibitor, when co-applied with D-serine, increased CSF concentration of D-serine in the rat to greater levels than D-serine alone. Whether this is due to the DAAO inhibitors acting on the enzyme in the periphery or the CNS is unclear, but raises the possibility that DAAO inhibitors may facilitate dosing patients with lower concentrations of D-serine, thereby avoiding possible undesirable effects of higher doses of D-serine.<sup>123,165,166</sup>

AS057278 is a brain-penetrant DAAO inhibitor which inhibits human recombinant DAAO enzyme with an  $IC_{50}$  of  $0.91 \mu\text{M}$ <sup>161</sup> and increases brain D-serine levels when administered systemically. Both acute dosing ( $80 \text{ mg kg}^{-1}$ ) and chronic dosing ( $20 \text{ mg kg}^{-1}$  bid, for 4 weeks but not 10 and  $40 \text{ mg kg}^{-1}$ ) of AS057278 normalized PCP-induced disruption of PPI in mice. Acute dosing had no effect on PCP-induced locomotion, whereas chronic dosing at 10 and



20 mg kg<sup>-1</sup> normalized the effects of PCP. Under the conditions tested, the compound exerted no significant effect on startle response, baseline PPI or locomotion.<sup>161</sup> The actions of AS057278 may be, however, independent of inhibition of DAAO modulation of D-serine levels; the compound attenuated conversion of infused D-kynurenine to kynurenic acid, an antagonist of NMDA<sup>161</sup> and alpha 7 nicotinic acetylcholine receptors<sup>167</sup> in the rat prefrontal cortex.<sup>168</sup> Whether this is a property of other DAAO inhibitors remains to be seen.

### 4.3 AMPA Receptor Modulators

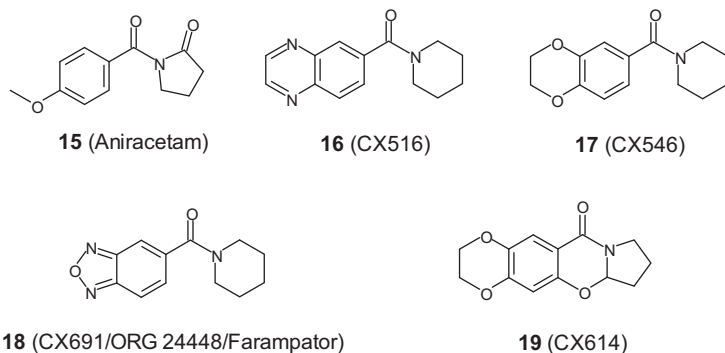
AMPA receptors mediate the majority of fast excitatory amino acid transmission in the CNS and participate in forms of synaptic plasticity thought to underlie learning and memory. Recently there has been a growing appreciation of the utility of positive allosteric modulators of the AMPA receptor as a means of increasing glutamatergic function while avoiding excitotoxic effects of direct agonists. Several classes of potent, selective and centrally active AMPA receptor positive modulators have now been described and have been shown to enhance synaptic transmission and long-term potentiation (LTP) and increase the expression of neurotrophic factors. AMPA receptor subunits are encoded by four separate genes (GluR1 to 4; also referred to as GluRA to D), each existing as two splice variants, termed flip and flop, conferring distinct kinetic and pharmacological properties on the channel.<sup>169,170</sup> Further diversity of the AMPA receptors arises from RNA editing, the most prominent being the Q/R site located in the pore region of the GluR2 subunit. The R variant, which the majority of native GluR2 subunits are believed to comprise, is characterized by significantly reduced calcium permeability. A further R/G editing site is located in the S2 domain of GluR2, GluR3 and GluR4 with the G form exhibiting accelerated recovery from desensitization.

AMPA receptor subunits co-assemble to form heterotetrameric complexes comprising an ion channel through which sodium, and (with the exception of receptors containing the edited GluR2 subunit) calcium ions enter the cell upon receptor activation (Figure 4.1). Collectively, the heterogeneity of AMPA receptor subunit expression across the brain, together with the differences conferred by heteromeric subunit combinations, RNA editing and splice variant composition, contribute to pharmacological as well as biophysical (*e.g.* kinetics of desensitization and deactivation) variability in the properties of AMPA receptors. In turn, this leads to considerable heterogeneity in AMPA-mediated synaptic responses across the brain. The kinetics of desensitization and deactivation are key functional properties of AMPA receptors that shape the amplitude and duration of synaptic responses to glutamate. Desensitization is the process of ion channel closure with agonist remaining bound to the receptor and occurs extremely rapidly and profoundly within 10 ms of receptor activation. Deactivation is the process of channel inactivation following the dissociation of glutamate and is typically measured as the decay of agonist-induced current following removal of agonist. Both desensitization and

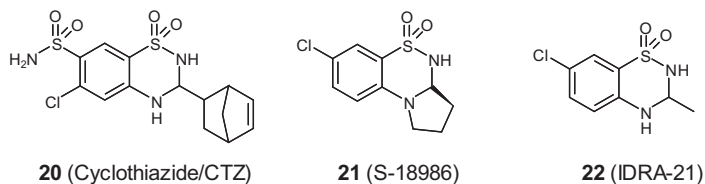
deactivation can be regulated by allosteric modulators of the AMPA receptor, which interact at a site remote from agonist binding and influence either agonist binding itself or the conformational changes associated with receptor gating and/or desensitization.<sup>171</sup>

The first AMPA positive allosteric modulators were discovered by researchers at the University of California who found that certain putative nootropic drugs such as Aniracetam were able to potentiate AMPA receptor-mediated currents in neuronal cultures.<sup>172</sup> Chemical modification and optimization led to the discovery of a number of related “benzamide” AMPA modulators (Figure 4.6). Comparison of the biological data on these and other AMPA modulators is not straightforward as the data are reported in various different cell and assay formats. However, early benzamides such as CX516 and CX546 are regarded as relatively weak potentiators ( $EC_{50} > 100 \mu M$ ).<sup>173</sup> Conformational constraint resulted in compounds such as CX614,<sup>174</sup> which has considerably improved potency relative to CX546.

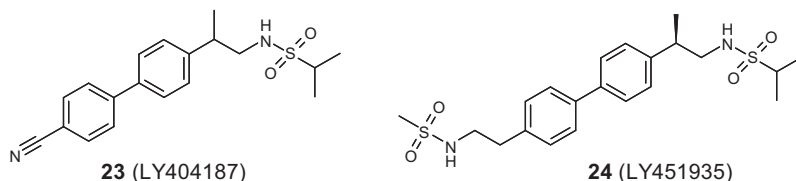
The benzothiadiazines (Figure 4.7) represent another early class of AMPA positive allosteric modulator. These compounds are derived from cyclothiazide (CTZ), which was originally developed as a diuretic agent and subsequently identified as an AMPA receptor positive allosteric modulator.<sup>175</sup> The best characterized examples from this class are IDRA-21<sup>176</sup> and S-18986,<sup>177</sup> which have been extensively profiled (see below).



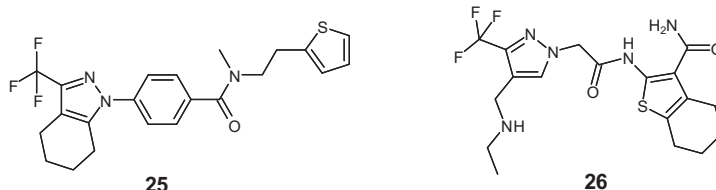
**Figure 4.6** Benzamide AMPA receptor modulators.



**Figure 4.7** Benzothiadiazine AMPA receptor modulators.



**Figure 4.8** Sulfonamide-derived AMPA receptor modulators.



**Figure 4.9** Pyrazole-based AMPA receptor modulators.

In addition to these early discoveries, high-throughput screening efforts by Lilly led to the identification of phenethyl sulfonamide AMPA receptor positive allosteric modulators, best exemplified by LY404187<sup>178</sup> and LY451395<sup>179</sup> (Figure 4.8).

More recently trifluoromethyl-substituted pyrazoles have emerged as another important class of AMPA receptor positive allosteric modulator. This chemotype has been exploited by groups from Merck and GSK and includes examples such as tetrahydroindazole derivative **25**<sup>180</sup> and pyrazole derivative **26** (Figure 4.9). Also see Chapter 5, Discovery and Clinical Data for a Novel AMPA Receptor Positive Modulator, Simon Ward.

### 4.3.1 Preclinical Studies with AMPA Receptor Modulators

AMPA receptor modulators have been shown to be active in a number of preclinical tests predictive for antipsychotic-like activity. Amongst the earliest studies was the demonstration that that AMPAkinine CX546 counteracted the stereotypic rearing behaviour elicited by methamphetamine in rats.<sup>181</sup> CX546 was also shown to have an opposing effect to methamphetamine on striatal c-fos activity (lowering c-fos as opposed to a marked increase with methamphetamine).<sup>182</sup> Another compound, CX516, failed to show any significant effect against methamphetamine or MK801-induced locomotor activity when given alone<sup>183,184</sup> but did enhance the inhibitory effect of clozapine and, to a lesser extent, haloperidol with a pharmacokinetic interaction being ruled out.<sup>184</sup> No exacerbation of haloperidol-induced catalepsy was noted indicating that the interaction was specific for the antipsychotic-like effect with a reduced likelihood for exacerbating extra-pyramidal side-effects. CX516 also significantly

potentiated the suppression of Conditioned Avoidance Responding (CAR) in the rat induced by threshold doses of risperidone, olanzapine and clozapine while being found to have no effect on its own.<sup>185</sup> CX546 and CX614 were other compounds demonstrated to reduce methamphetamine-induced stereotypic behaviour (circling behaviour in rats with unilateral 6-hydroxydopamine lesions of the ascending nigro-striatal dopamine system).<sup>186</sup>

While evidence supporting the potential of AMPA-receptor positive modulators as antipsychotics is limited, the prominent role that AMPA receptors have been demonstrated to play in forms of synaptic plasticity *e.g.* LTP, underlying memory encoding, it may be anticipated that these compounds will serve as a promising adjunctive therapy for cognitive impairment in schizophrenia. The positive effects of a number of these compounds have been well documented over the last few years across a variety of cognitive domains in both rodent and non-human primates in normal and deficit models.<sup>187–190</sup> Collectively, these data show that multiple classes of AMPA-receptor positive modulators such as the benzamides (aniracetam, CX516, CX546), benzothiadiazines (IDRA-21, S18986) and biarylsulfonamides (LY404187, LY451395) facilitate memory performance in a number of preclinical tests. Furthermore, a number of these tests fall into categories that may map onto cognitive domains that are impaired in schizophrenia as identified by the MATRICS consortium, with for example Delayed Non Match to Sample (DNMS), novel object recognition and maze tasks potentially mapping onto cognitive domains such as working memory, visual learning and memory, and reasoning and problem solving, respectively.<sup>191</sup> However, caution is required in the interpretation of these results since they have been derived, for the most part, in experiments with healthy animals, some with age-related or sleep-deprivation-induced cognitive deficits. None of these can really be considered as models of schizophrenia and, indeed, the neurobiological substrates underlying the deficits resulting from old age or sleep deprivation may well be different from those present in schizophrenia. There is a genuine need for development of animal models that express a deficit that resembles that seen in the patients, *i.e.* some level of face and construct validity. In this respect, animal models involving sub-chronic PCP treatment may be of interest since they appear to mimic several symptoms domains of schizophrenia including cognitive deficits in models such as reversal learning,<sup>192</sup> attentional set shifting<sup>193</sup> and novel object recognition.<sup>194</sup> Indeed, the demonstration that both CX516 and CX547 were able to reverse sub-chronic PCP-induced disruption of object memory in the novel object recognition task<sup>195</sup> provides some evidence that AMPA modulation may represent a mechanism for the treatment of cognitive deficits in schizophrenia.

### 4.3.2 Clinical Studies with AMPA Receptor Modulators

The potential therapeutic utility of AMPA receptor modulators is being explored for a number of indications including schizophrenia.<sup>190,196–200</sup> Clinical data, however, are still quite limited with only a few compounds, CX516,

CX717, Org 24448 (farampator) and LY451395, having progressed to phase II trials. Indeed, of these, the only AMPA-positive modulator with published clinical data for schizophrenia is CX516, a relatively low-potency compound with sub-optimal pharmacokinetic properties. Early studies with this compound in human volunteers provided encouraging data to suggest that it would have a positive effect on memory performance. A transient improvement in the delayed recall of nonsense syllables was demonstrated following administration of a single 900 mg dose of CX516 in elderly subjects (aged >65 years)<sup>201</sup> and following single CX516 doses ranging from 600–1200 mg in younger subjects.<sup>202</sup> CX516 also produced small, but statistically significant, improvements in a series of cognitive tasks measuring visual, olfactory and visiospatial memory following repeated administration of 300 mg in a group of healthy males (aged 25–35 years).<sup>203</sup> To date, three double-blind studies have been published on the clinical effects of CX516 in schizophrenia. A limited study by Marenco *et al.*<sup>204</sup> failed to observe any antipsychotic effect of CX516 monotherapy at dosages of between 300 and 900 mg three times daily for 2–4 weeks on four partially refractory schizophrenia patients withdrawn from antipsychotic treatment. More encouraging data were initially provided by Goff *et al.*<sup>205</sup> who reported on a preliminary 4-week placebo-controlled add-on study in 19 patients treated with clozapine. CX516 at doses of 900–1200 mg three times daily was associated with improvements in measures of attention and memory with the effects persisting for up to two weeks after completion of the trial. However, a more comprehensive follow-up study examining the effect of add-on treatment with CX516 (900 mg three times daily) or placebo on a total of 105 schizophrenia patients treated with clozapine, olanzapine or risperidone failed to show any effect on any measure of cognition or for symptoms of schizophrenia.<sup>206</sup> Further development of CX516 has since been terminated and results from studies with more optimal compounds are eagerly awaited. Of the other compounds to reach clinical development, farampator (500 mg) improved working memory and information processing in a double-blind placebo-controlled study performed with 16 healthy, elderly volunteers but appeared to impair episodic memory.<sup>207</sup> Following up on the demonstration that CX717 was able to improve cognitive performance in sleep-deprived monkeys,<sup>208</sup> Cortex Pharmaceuticals reported that CX717 had an alerting effect on sleep deprivation trials in healthy young men and produced some attenuation of cognitive deficits. However, in a double-blind placebo-controlled trial in 48 volunteers undergoing four consecutive nights of simulated night shift work, CX717 was not effective in reversing performance and alertness deficits when tested at doses between 200 and 1000 mg.<sup>209</sup> And finally, LY451395 (administered 0.2 mg bid for 28 days and 1.0 mg bid thereafter up to a maximum of 8 weeks) failed to have any effect on cognitive outcome in a phase II clinical study on 181 patients with Alzheimer's disease although it did show improvements in neuropsychiatric symptoms as measured by the Neuropsychiatric Inventory (NPI) rating scale.<sup>210</sup>

Overall, the clinical studies that have been performed thus far have delivered mixed results. While there are some promising data to suggest that AMPA

receptor positive modulators may have some utility to improve cognition, the precise reasons for the apparent lack of efficacy in a number of these studies is presently unknown and may be due to a number of factors. Some compounds (CX516 for example) have suboptimal pharmacokinetic properties and are probably not the best tools to establish proof-of-concept for AMPA-receptor positive modulators in the clinic. In other cases, as suggested by the authors of the study with LY451395,<sup>210</sup> the doses that have been used may not have been optimal. Indeed, perhaps one of the key issues facing clinical development of AMPA-receptor modulators is the lack of suitable biomarkers for dose prediction in man. While measuring drug levels in CSF may give an indication of exposure in the brain, proof of target engagement (*i.e.* interaction of the drug with the AMPA receptor) is still lacking. Although PET ligands to measure receptor occupancy do not yet exist for the AMPA receptor,<sup>211</sup> the use of indirect measures such as pharmacological magnetic resonance imaging (phMRI), as used to demonstrate the dose-dependent central effects of LY404187 on neuronal activity in specific brain regions in rodents, may prove valuable.<sup>212</sup>

## 4.4 Metabotropic Glutamate Receptors

Functional metabotropic glutamate (mGlu) receptors were first identified in the 1980s, with the first cloned mGlu receptor, mGlu1, reported in 1991, followed by subsequent identification of an additional seven related receptor genes. On the basis of sequence homology, pharmacology and signal transduction mechanism, the eight receptors are divided into three groups: group I contains receptors mGlu1 and mGlu5; group II receptors mGlu2 and mGlu3 with group III containing mGlu4, mGlu6, mGlu7 and mGlu8. Within each group, the receptors share about 70% sequence homology with 45% shared overall.<sup>213,214</sup>

The group I receptor mGlu5 and group II receptors are of particular interest as targets for the treatment of schizophrenia and have been the subject of several recent reviews.<sup>215–217</sup>

### 4.4.1 Group II mGlu Receptors

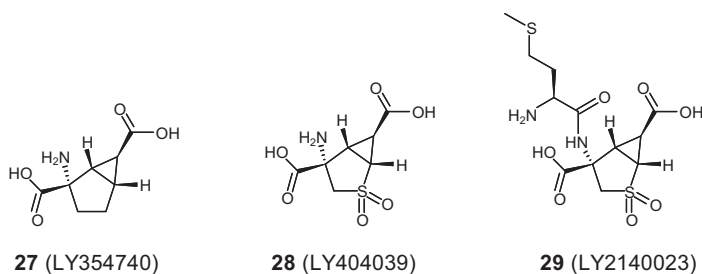
The genes encoding human group II mGlu receptors, mGlu2 (GRM2) and mGlu3 (GRM3), map to chromosomes 3 (p21.1)<sup>218</sup> and 7 (7q21.1-q21.2),<sup>219</sup> respectively, and there is some evidence from genetic association studies that mGlu3 receptor polymorphisms co-segregate with schizophrenia and deficits in cognitive function<sup>220–223</sup> (although see <sup>224,225</sup> for conflicting findings). There is also evidence of altered mGlu3 receptor expression in schizophrenia,<sup>226</sup> with a reported reduction in levels of mGlu3 receptor dimers.<sup>227</sup> In contrast, mGlu2 receptor polymorphisms do not appear to be predictive of a schizophrenic phenotype,<sup>228,229</sup> although there may be a decrease in mGlu2 receptor levels in schizophrenics.<sup>230</sup> However, all other evidence (discussed below) suggests that it is modulation of the mGlu2 receptor rather than mGlu3 that is likely to be of therapeutic benefit.

Group II receptors are expressed in the forebrain and limbic systems with mGlu3 receptor more widely distributed throughout the brain compared to

mGlu2. Both receptors are expressed in neurons, both pre- and post-synaptically, and in extra-synaptic locations.<sup>231,216</sup> The mGlu2 and mGlu3 receptors have also been identified in a number of peripheral tissues, including the kidney and gastrointestinal tract (for references see <sup>232</sup>).

The rationale for targeting group II mGlu receptors for the treatment of schizophrenia is supported by a number of preclinical and clinical studies with small-molecule agonists and modulators of group II mGlu receptors. Early endeavours focused on the identification of orthosteric agonists such as LY354740<sup>233</sup> and LY404039<sup>234</sup> (Figure 4.10). These compounds are constrained analogues of L-glutamate and bind the orthosteric binding site located on the large extra-cellular N-terminal domain of the receptor. LY35470 and LY404039 exhibit no agonist or antagonist activity at group I and III receptors at concentrations of up to 100  $\mu$ M, but potently activate both mGlu2 and mGlu3 at nanomolar concentrations. These compounds show limited selectivity between mGlu2 and mGlu3,<sup>234,233</sup> reflecting the highly conserved sequence homology of the mGlu2 and mGlu3 receptor binding site,<sup>217</sup> and to date no subtype selective orthosteric agonists have been reported. The prerequisite similarity to L-glutamate also renders these small-molecule agonists non-“drug like” and, as a result of their highly polar, charged nature, they are poorly absorbed. However, it was found that LY2140023 (Figure 4.10), the methionine amide of LY404039, acts as a pro-drug resulting in significantly improved absorption.

In preclinical studies, LY354740 (10 mg kg<sup>-1</sup> ip) abolished PCP-induced efflux of glutamate in the prefrontal cortex and attenuated the effects of PCP on working memory and locomotor activity.<sup>235</sup> Subsequent studies have reported similar effects of group II agonists in models of schizophrenia (see <sup>217</sup>). In clinical studies, LY2140023 has advanced to phase III trials ([www.clinical-trials.gov](http://www.clinical-trials.gov)) and has been reported to produce statistically significant improvement in positive and negative symptoms in a three-armed, double-blind, placebo-controlled study (118 patients completing the trial; LY2140023 dose 40 mg twice daily for 4 weeks). LY2140023 was found to be safe and well tolerated, exhibiting a rapid onset of action with effects being observed within the first week of treatment.<sup>236</sup> However, in a subsequent study, the results were deemed inconclusive with no significant effect being observed with either



**Figure 4.10** Orthosteric mGluR2/3 agonists.



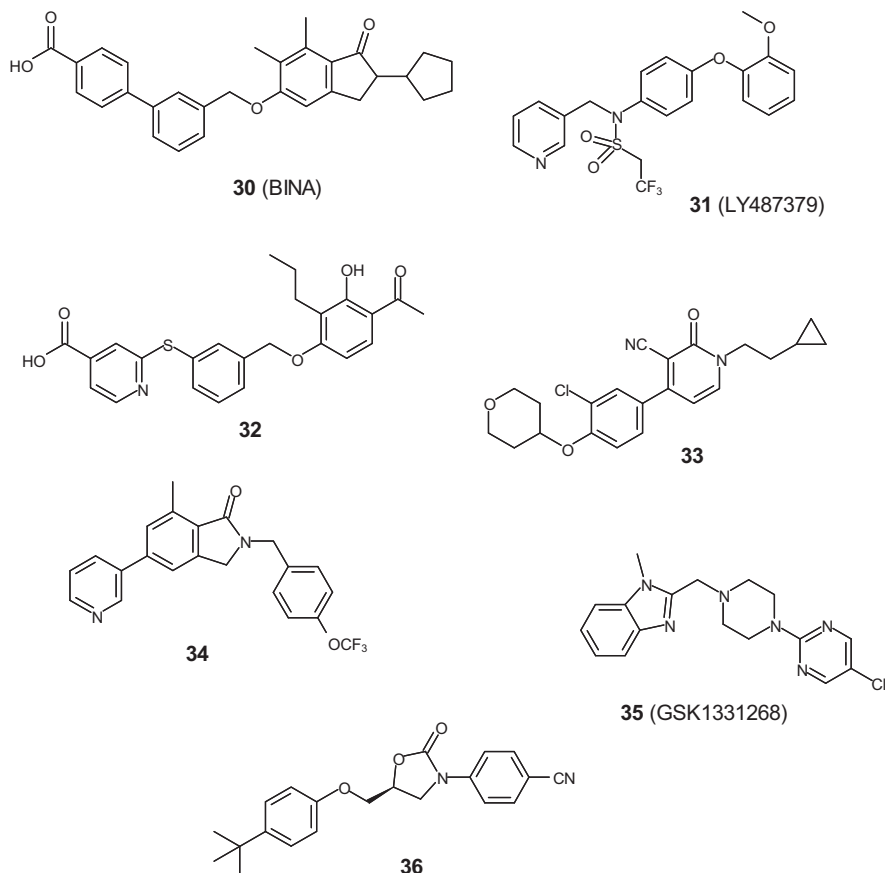
LY2140023 or the active control olanzapine; however, an unexpectedly high placebo response was observed, perhaps accounting for the apparent lack of clinical effect.<sup>237</sup> In this study, four patients in the LY2140023 group suffered convulsions (five events in total); however, convulsions have not been reported in previous clinical studies with group II agonists,<sup>238,236</sup> and the occurrence of these events did not appear to associate with the dosing or timing of drug administration.<sup>237</sup>

Studies in mutant mice lacking either mGlu2 or mGlu3 suggest that it is the former, rather than the latter, that mediates the therapeutically beneficial effects of group II mGlu agonists.<sup>239</sup> The identification of mGlu2 receptor positive allosteric modulators (PAMs)<sup>215</sup> led the development of compounds that are highly selective for mGlu2 with a number of potential advantages over the direct agonism approach. PAMs can be as efficacious as orthosteric agonists, with improved selectivity and safety profile and lower potential for development of tolerance compared to full agonists.<sup>217</sup> From a medicinal chemistry perspective there is an enhanced prospect of identifying subtype-selective, drug-like, non-amino acid derivatives as allosteric modulators can bind sites where the sequence homology is less conserved. Amongst the earliest characterized compounds of this class were BINA (**30**)<sup>240,241</sup> and LY487379 (**31**) (Figure 4.11).<sup>242</sup> Both are selective mGlu2 PAMs and bind to regions spatially distinct from the glutamate binding region. Point mutation studies have demonstrated that both compounds bind a distinct pocket of mGlu2, with amino acids crucial for PAM efficacy being identified in transmembrane regions IV (Ser688 or Gly689) and V (Asn735).<sup>243,244</sup>

BINA belongs to the indanone class of mGlu2 PAMs and a number of related compounds have been described, including arylketones, pyridones and isoindolones such as **32**,<sup>245</sup> **33**<sup>246</sup> and **34**,<sup>247</sup> respectively (Figure 4.11). Structurally distinct compounds such as the benzimidazole **35** (GSK1331268)<sup>248</sup> and oxizolidinone **36**<sup>249</sup> have been reported more recently (Figure 4.11). The most advanced mGlu2 PAM appears to be ADX-71149 (structure not disclosed), which has been reported to have progressed to phase II clinical studies for the treatment of schizophrenia (Addex press release, March 2011).

These PAMs possess negligible inherent activity but cause a leftward shift in the concentration response curve of agonists such as glutamate. BINA, for example, produces up to an 11-fold parallel leftward shift in the EC<sub>50</sub> of glutamate and, when tested in the presence of a fixed concentration of glutamate (EC<sub>20</sub>), BINA has an EC<sub>50</sub> less than 100 nM, with no apparent effect on the mGlu3 receptor subtype.<sup>240</sup> BINA also enhanced the effects of agonists in native tissue preparations, and in rodent behavioural models, attenuated the effects of an hallucinogenic 5-HT<sub>2A/2C</sub> receptor agonist<sup>250</sup> and PCP on PPI and reduced the PCP-induced increases in blood oxygenation level-dependent activation in brain areas of relevance to schizophrenia, including the cortex, thalamus and limbic regions of anaesthetized rats.<sup>240,251</sup> BINA was, however, ineffective on amphetamine-induced hyperlocomotor activity<sup>240</sup> and in this respect differs from orthosteric agonists and the mGlu2 receptor PAM LY487379.<sup>252</sup> LY487379 produces a more modest two-fold shift in agonist

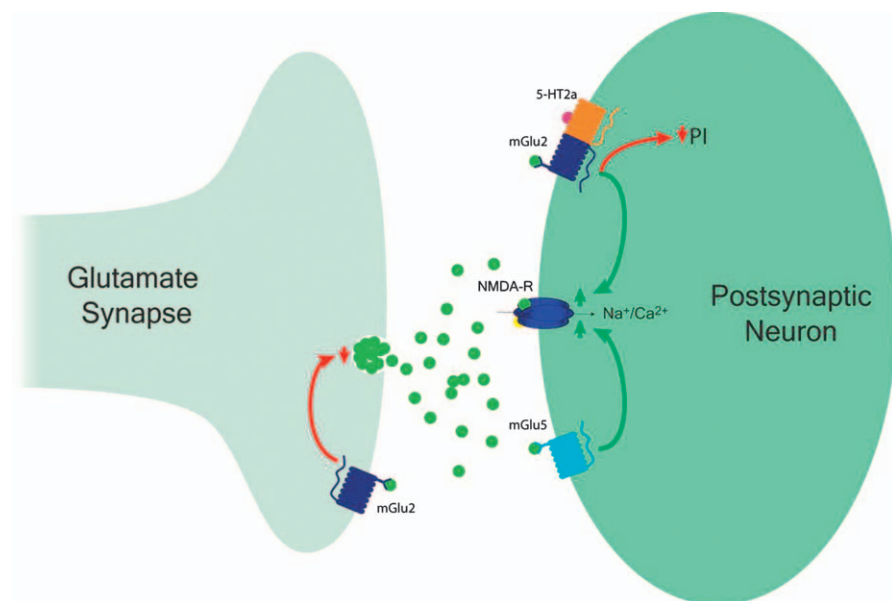




**Figure 4.11** mGlu2 positive allosteric modulators.

potency, but, as with BINA, exhibits no activity at other mGlu receptor subtypes.<sup>242</sup> LY487379 was active in a range of behavioural tests of relevance to schizophrenia, including PCP- and amphetamine-induced hyperlocomotor activity in mice,<sup>252</sup> deficits in social discrimination induced by neonatal treatment of rats with PCP<sup>82</sup> and procognitive effects in rats assessed in an attentional set-shifting task.<sup>253</sup>

Activation of post-synaptic mGlu2 receptors has been reported to enhance NMDA receptor function *via* the Akt/GSK-3 $\beta$  pathway<sup>254</sup> thereby attenuating the effects of NMDA receptor antagonism. However, mGlu2 receptors may operate by alternative or additional mechanisms (Figure 4.12). Increased levels of glutamate have been reported in schizophrenic patients and PCP and other hallucinogenic drugs have been shown to enhance glutamate release.<sup>255</sup> At many synapses, group II mGlu receptors are located pre-synaptically where they function as auto-receptors to suppress release of glutamate.<sup>256</sup> Therefore, mGlu2 receptor-mediated suppression of glutamate release has been suggested



**Figure 4.12** Actions of mGlu receptors on synaptic transmission that may underlie the beneficial effects on the symptoms of schizophrenia produced by modulating these receptors. NMDA receptors are activated by glutamate released from the pre-synaptic terminal. Activation of post-synaptic mGlu5 receptors is known to increase the signal produced by the NMDA receptors. The mGlu2 receptors exert multiple effects on neuronal function. Pre-synaptic receptors modulate release of glutamate, and other neurotransmitters. Post-synaptic mGlu2 receptors modulate NMDA receptor signalling and form functional dimers with 5HT<sub>2A</sub> receptors and modulate activity of this receptor.

as a mechanism of action.<sup>257,258</sup> A further intriguing possibility arises from a body of data describing an interaction between mGlu2 receptors and 5-HT<sub>2A</sub> receptors. These receptors form functional complexes in neuronal tissue,<sup>230</sup> and it has been demonstrated that 5-HT<sub>2A</sub> receptor-induced polyphosphoinositide hydrolysis<sup>259</sup> and the effects of 5-HT<sub>2A</sub> agonists in behavioural and electrophysiological studies can be modulated by mGlu2 receptors.<sup>260,261</sup> The behavioural effects of 5-HT<sub>2A</sub> activation are also absent in mGlu2 receptor knockout mice.<sup>262</sup> Evidence that this interaction may be relevant to the treatment of schizophrenia stems from an analysis of DNA samples collected from clinical trials of LY2140023, which concluded that genetic variants of the 5-HT<sub>2A</sub> receptor were associated with responsiveness to LY2140023.<sup>263</sup>

#### 4.4.2 Group I mGlu Receptors

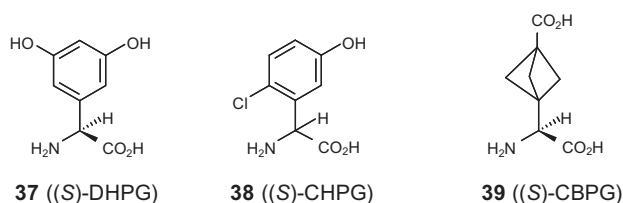
The group I mGlu receptor family consists of two receptors, mGlu1 and mGlu5, which typically couple to G<sub>q/11</sub> leading to stimulation of phospholipase

C and phosphoinositide hydrolysis.<sup>264</sup> The mGlu5 gene, GRM5, has been mapped to 11q14.3 and codes for a protein of 1212 amino acids.<sup>265</sup> The mGlu5 receptors are present in a range of brain areas including hippocampus, cerebral cortex, thalamus and cerebellum and are located predominately in a peri-synaptic location on the post-synaptic membrane<sup>266–269</sup> (Figure 4.12), although functional pre-synaptic receptors have also been reported.<sup>270</sup> The mGlu5 receptor has also been identified in a number of peripheral tissues (see<sup>232</sup> for references) and mGlu5 receptor antagonists and are under development for treatment of gastroesophageal reflux.<sup>271</sup>

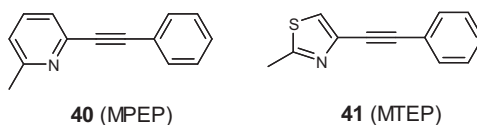
There is evidence suggesting that modulation of either group I receptor subtype may be of potential benefit for the treatment of schizophrenia. While antagonism of mGlu1 has been reported to result in antipsychotic-like effects in rodents,<sup>272–274</sup> much greater attention has been focused on the mGlu5 receptor. A possible link between the mGlu5 gene and schizophrenia has been identified in an association study<sup>265</sup> and there is evidence that mGlu5 receptor levels are altered in the schizophrenic brain.<sup>275,276</sup> Preclinical support for mGlu5 as a target for schizophrenia arises from evidence that the receptor is functionally coupled to the NMDA receptor (Figure 4.12), and that activation of mGlu5 modulates cognitive behaviour and ameliorates symptoms in rodent models of schizophrenia.<sup>217</sup> A number of orthosteric agonists of the mGlu5 receptor have been identified (Figure 4.13). These compounds are non-drug-like amino acid derivatives that are somewhat limited as tools to study mGlu5 receptor function: they are either non-selective between mGlu1 and mGlu5 (*e.g.* (*S*)-3,5-dihydroxyphenylglycine; DHPG<sup>277</sup>), are only weakly active (*e.g.* (*RS*)-2-chloro-5-hydroxyphenylglycine; CHPG<sup>278</sup>) or exhibit other actions, such as mGlu1 receptor antagonism (*e.g.* (*S*)-(+)-2-(3'-carboxybicyclo(1.1.1)pentyl)-glycine; CBPG).<sup>279</sup>

The discovery of mGlu5 allosteric modulators (positive and negative) has resulted in the availability of a number of non-amino acid drug-like compounds. Amongst the first to be described were the mGlu5 receptor-selective antagonists 2-methyl-6-(phenylethynyl)pyridine (MPEP<sup>280</sup>) and 3-((2-methyl-1,3-thiazol-4-yl)ethynyl)pyridine (MTEP<sup>281</sup>) shown in Figure 4.14. These two structurally related compounds function as negative modulators of the receptor and bind to a region distinct from the orthosteric agonist binding site.<sup>282,283</sup>

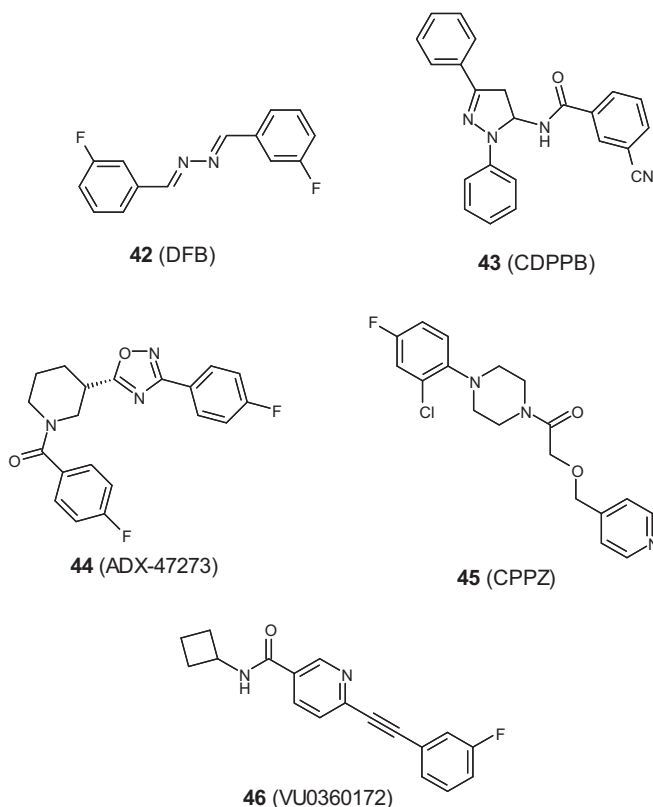
More significantly, a number of potent and selective mGlu5 PAMs have been identified (Figure 4.15), including: 3,3'-difluorobenzaldazine (DFB),<sup>284</sup>



**Figure 4.13** mGlu5 receptor orthosteric agonists.



**Figure 4.14** mGlu5 receptor allosteric antagonists.



**Figure 4.15** mGlu5 receptor positive allosteric modulators.

3-cyano-*N*-(1,3-diphenyl-1*H*-pyrazol-5-yl)benzamide (CDPPB),<sup>285</sup> *N*-[4-chloro-2-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]phenyl]-2-hydroxybenzamide (CPPHA),<sup>286</sup> [*S*-(4-fluorophenyl)-{3-[3-fluorophenyl]-[1,2,4]-oxadiazol-5-yl]-piperidin-1-yl}-methanone] (ADX47273),<sup>287</sup> *N*-cyclobutyl-6-[(3-fluorophenyl)ethynyl]nicotinamide hydrochloride (VU0360172)<sup>288</sup> and 1-(4-(2-chloro-4-fluorophenyl)piperazin-1-yl)-2-(pyridin-4-ylmethoxy)ethanone (CPPZ).<sup>289</sup> All mGlu5 PAMs have similar functional properties in that they exhibit little, if any, intrinsic agonist activity, but enhance the effect of glutamate at the receptor. However, different PAMs appear to act at distinct binding

sites,<sup>290,286,291</sup> and show some differences in mechanism of action, with DFB and CDPPB modifying affinity for the orthosteric agonist, whereas others, such as ADX47273, act primarily by modulating agonist efficacy.<sup>292</sup> It not yet clear whether the differing modes of action offer any therapeutic advantage. Some of the more recently discovered compounds, such as VU0360172,<sup>288</sup> are both orally available and brain penetrant.

NMDA and mGlu5 receptors are tethered together in the post-synaptic density<sup>213</sup> and a number of studies have demonstrated enhanced NMDA receptor activity in neurons in the presence of mGlu5 receptor agonists and PAMs.<sup>213,217</sup> The ability of mGlu5 receptor PAMs to enhance NMDA receptor function suggests they may be able to alleviate symptoms of schizophrenia by attenuation of the effects of NMDA receptor hypofunction,<sup>24</sup> and a number of preclinical studies suggest mGlu5 receptor PAMs may indeed be efficacious in multiple-symptom domains. Activation of mGlu5 results in modulation of LTP (see <sup>293</sup> for references) and a greater magnitude of LTP is induced in the presence of mGlu5 PAMs.<sup>289,294,295</sup> Consistent with the effects on hippocampal LTP, mice dosed with the CDPPB, ADX47273 and DFB showed improved spatial learning performance in the Morris water maze and Y-maze tasks,<sup>294,296</sup> with MK-801-induced deficits in cognitive performance in active allothetic place avoidance ameliorated by mGlu5 receptor activation.<sup>297</sup> ADX47273 and CDPPB have also been shown to improve recognition memory in normal rats as well as reversing MK-801-induced deficits in performance.<sup>287,298</sup> In the same study,<sup>298</sup> CDPPB administration was associated with increased levels of phosphorylated cAMP responsive element-binding protein (CREB), which is critical to memory formation in many species, including humans.<sup>299,300</sup> In set-shifting tests of cognitive flexibility, mGlu5 receptors were found to play no role in normal performance, but CDPPB dosing reduced the impaired performance produced by MK-801.<sup>301</sup> Mutant mice lacking the mGlu5 receptor show deficits in sensorimotor gating reflected in a reduction of PPI assessed across a variety of sensory modalities,<sup>302,303</sup> and when PPI is disrupted with a variety of agents, the disruption of PPI is exacerbated when mGlu5 receptor antagonists are co-administered, and agonists and PAMs ameliorate the disruption of PPI.<sup>213,217</sup>

Indirect modulation of NMDA receptors *via* mGlu5 receptor PAMs may offer fewer side-effect liabilities than more direct activation of NMDA and may also prove to have advantages in terms of side-effects and tachyphylaxis over orthosteric agonists. Indeed, repeated dosing of CDPPB over 7 days produced no evidence of tolerance to the antipsychotic effect observed on amphetamine-induced hyperlocomotion in rats, although tolerance did develop to effects on sleep architecture.<sup>304</sup>

## 4.5 Conclusion

The glutamate theory of schizophrenia was first proposed over two decades ago based on observations with drugs such as ketamine and PCP, whose powerful psychotomimetic effects are mediated by blockade of the NMDA-subtype of

ionotropic glutamate receptor. Since then, a whole host of pharmacological approaches for the treatment of schizophrenia have been developed by the pharmaceutical industry based on potentiation of glutamatergic neurotransmission. Despite considerable effort by a number of companies, it is still premature to say if any of these approaches will prove effective. Early clinical studies with sub-optimal pharmacological agents such as glycine, D-serine and sarcosine provided some encouragement but it has only been recently that new treatment approaches have been entering more advanced stages of clinical development. Of these, the most significant are Roche's GlyT1 inhibitor RG1678 and Eli Lilly's mGluR2/3 agonist LY2140023, both currently in phase III. Both drugs appear to have promise for addressing key unmet medical needs in schizophrenia with RG1678 showing efficacy against negative symptoms, and LY2140023 proving effective against positive and negative symptoms without any of the tolerability issues associated with SGAs. Still, the phase II clinical data for both drugs has proved somewhat equivocal and it will only be with the completion and publication of the ongoing phase III studies that it will be determined if these approaches will indeed provide a breakthrough in the treatment of schizophrenia. With GlyT1 inhibitors, side-effects mediated by potentiation of the strychnine-sensitive glycine receptor remain a concern and, in this respect, approaches focusing on elevation of brain D-serine levels (DAAO and ASC1 inhibitors, respectively) with their selective modulation of NMDA-mediated neurotransmission may prove to have an advantage. Similarly, positive modulation of mGluR5, given its functional coupling to NMDA receptors is another promising approach although no such compounds have yet reached clinical development. AMPA receptor modulators have the potential to treat cognitive deficits associated with schizophrenia and those that have entered clinical development are better tolerated than would have been expected given the link with excitotoxicity that has been associated with excessive activation of ionotropic glutamate receptors. While preliminary data have again proved equivocal, the expectation is that compounds with an optimal balance between efficacy, safety and pharmacokinetic properties will have the potential to provide a breakthrough. Thus, glutamatergic based approaches offer particular hope for treatment of the key unmet medical needs in schizophrenia, negative symptoms and cognitive deficits in particular, and bring improvement to the lives of millions of patients worldwide who suffer from this devastating disease.

## References

1. K. H. Nuechterlein, D. M. Barch, J. M. Gold, T. E. Goldberg, M. F. Green and R. K. Heaton, *Schizophr. Res.*, 2004, **72**, 29.
2. M. F. Green, B. Olivier, J. N. Crawley, D. L. Penn and S. Silverstein, *Schizophr. Bull.*, 2005, **31**, 882.
3. M. F. Green, K. H. Nuechterlein, J. M. Gold, D. M. Barch, J. Cohen, S. Essock, W. S. Fenton, F. Frese, T. E. Goldberg, R. K. Heaton, R. S. E.

- Keefe, R. S. Kern, H. Kraemer, E. Stover, D. R. Weinberger, S. Zalcmán and S. R. Marder, *Biol. Psychiatry*, 2004, **56**, 301.
4. A. Carlsson, L. O. Hansson, N. Waters and M. L. Carlsson, *Life Sci.*, 1997, **61**, 75.
  5. M. Laruelle, C. D. D'souza, R. M. Baldwin, A. Abi-Dargham, S. J. Kanes, C. L. Fingado, J. P. Seibyl, S. S. Zoghbi, M. B. Bowers, P. Jatlow, D. S. Charney and R. B. Innis, *Neuropsychopharmacology*, 1997, **17**, 162.
  6. M. Laruelle and A. Abi-Dargham, *J. Psychopharmacol.*, 1999, **13**, 358.
  7. M. Laruelle, A. Abi-Dargham, R. Gil, L. Kegeles and R. Innis, *Biol. Psychiatry*, 1999, **46**, 56.
  8. M. Laruelle, A. Abi-Dargham, C. van Dyck, R. Gil, D. C. D'souza, J. Krystal, J. Seibyl, R. Baldwin and R. Innis, *Biol. Psychiatry*, 2000, **47**, 371.
  9. M. Laruelle, A. Abi-Dargham, C. H. van Dyck, R. Gil, C. D. D'souza, J. Erdos, E. McCance, W. Rosenblatt, C. Fingado, S. S. Zoghbi, R. M. Baldwin, J. P. Seibyl, J. H. Krystal, D. S. Charney and R. B. Innis, *Proc. Natl Acad. Sci. USA*, 1996, **93**, 9235.
  10. R. S. Keefe, R. M. Bilder, P. D. Harvey, S. M. Davis, B. W. Palmer, J. M. Gold, H. Y. Meltzer, M. F. Green, D. D. Miller, J. M. Canive, L. W. Adler, T. C. Manschreck, M. Swartz, R. Rosenheck, D. O. Perkins, T. M. Walker, T. S. Stroup, J. P. McEvoy and J. A. Lieberman, *Neuropsychopharmacology*, 2006, **31**, 2033.
  11. R. Rosenheck and D. Leslie, *Ment. Health Serv. Res.*, 2003, **5**, 149.
  12. J. A. Lieberman, T. S. Stroup, J. P. McEvoy, M. S. Swartz, R. A. Rosenheck, D. O. Perkins, R. S. Keefe, S. M. Davis, C. E. Davis, B. D. Lebowitz, J. Severe and J. K. Hsiao, *N. Engl. J. Med.*, 2005, **353**, 1209.
  13. P. B. Jones, T. R. Barnes, L. Davies, G. Dunn, H. Lloyd, K. P. Hayhurst, R. M. Murray, A. Markwick and S. W. Lewis, *Arch. Gen. Psychiatry*, 2006, **63**, 1079.
  14. L. M. Davies, S. Lewis, P. B. Jones, T. R. Barnes, F. Gaughran, K. Hayhurst, A. Markwick and H. Lloyd, *Br. J. Psychiatry*, 2007, **191**, 14.
  15. D. C. Henderson, *J. Clin. Psychiatry*, 2007, **68**(12), 18.
  16. H. A. Nasrallah, *Mol. Psychiatry*, 2008, **13**, 27.
  17. F. Schlagenhauf, G. Juckel, M. Koslowski, T. Kahnt, B. Knutson, T. Dembler, T. Kienast, J. Gallinat, J. Wrase and A. Heinz, *Psychopharmacology (Berl.)*, 2008, **196**, 673.
  18. R. S. Keefe, R. M. Bilder, S. M. Davis, P. D. Harvey, B. W. Palmer, J. M. Gold, H. Y. Meltzer, M. F. Green, G. Capuano, T. S. Stroup, J. P. McEvoy, M. S. Swartz, R. A. Rosenheck, D. O. Perkins, C. E. Davis, J. K. Hsiao and J. A. Lieberman, *Arch. Gen. Psychiatry*, 2007, **64**, 633.
  19. R. W. Buchanan, R. Freedman, D. C. Javitt, A. Abi-Dargham and J. A. Lieberman, *Schizophr. Bull.*, 2007, **33**, 1120.
  20. J. T. Coyle and G. Tsai, *Int. Rev. Neurobiol.*, 2004, **59**, 491.
  21. J. T. Coyle, G. Tsai and D. Goff, *Ann. NY Acad. Sci.*, 2003, **1003**, 318.
  22. J. H. Krystal, L. P. Karper, J. P. Seibyl, G. K. Freeman, R. Delaney, J. D. Bremner, G. R. Heninger, M. B. Bowers, Jr and D. S. Charney, *Arch. Gen. Psychiatry*, 1994, **51**, 199.



23. M. J. Millan, *Psychopharmacology*, 2005, **179**, 30.
24. C. G. Hahn, H. Y. Wang, D. S. Cho, K. Talbot, R. E. Gur, W. H. Berrettini, K. Bakshi, J. Kamins, K. E. Borgmann-Winter, S. J. Siegel, R. J. Gallop and S. E. Arnold, *Nat. Med.*, 2006, **12**, 824.
25. D. C. Javitt, I. Zylberman, S. R. Zukin, U. Heresco-Levy and J. P. Lindenmayer, *Am. J. Psychiatry*, 1994, **151**, 1234.
26. U. Heresco-Levy, D. C. Javitt, M. Ermilov, C. Mordel, A. Horowitz and D. Kelly, *Br. J. Psychiatry*, 1996, **169**, 610.
27. U. Heresco-Levy, D. C. Javitt, R. Ebstein, A. Vass, P. Lichtenberg, G. Bar, S. Catinari and M. Ermilov, *Biol. Psychiatry*, 2005, **57**, 577.
28. D. C. Goff, G. Tsai, D. S. Manoach and J. T. Coyle, *Am. J. Psychiatry*, 1995, **152**, 1213.
29. D. C. Goff, G. Tsai, J. Levitt, E. Amico, D. Manoach, D. A. Schoenfeld, D. L. Hayden, R. McCarley and J. T. Coyle, *Arch. Gen. Psychiatry*, 1999, **56**, 21.
30. D. C. Goff, L. Herz, T. Posever, V. Shih, G. Tsai, D. C. Henderson, O. Freudenreich, A. E. Evins, I. Yovel, H. Zhang and D. Schoenfeld, *Psychopharmacology*, 2005, **179**, 144.
31. H. Y. Lane, Y. C. Chang, Y. C. Liu, C. C. Chiu and G. E. Tsai, *Arch. Gen. Psychiatry*, 2005, **62**, 1196.
32. H. Y. Lane, Y. C. Liu, C. L. Huang, Y. C. Chang, C. H. Liao, C. H. Perng and G. E. Tsai, *Biol. Psychiatry*, 2008, **63**, 9.
33. D. C. Javitt, *Biol. Psychiatry*, 2008, **63**, 6.
34. R. W. Buchanan, D. C. Javitt, S. R. Marder, N. R. Schooler, J. M. Gold, R. P. McMahon, U. Heresco-Levy and W. T. Carpenter, *Am. J. Psychiatry*, 2007, **164**, 1593.
35. J. W. Johnson and P. Ascher, *Nature*, 1987, **325**, 529.
36. H. Betz, *Neuron*, 1990, **5**, 383.
37. J. Guastella, N. Brecha, C. Weigmann, H. A. Lester and N. Davidson, *Proc. Natl Acad. Sci USA*, 1992, **89**, 7189.
38. B. Borowsky, E. Mezey and B. J. Hoffman, *Neuron*, 1993, **10**, 851.
39. K. M. Kim, S. F. Kingsmore, H. Han, T. L. Yang-Feng, N. Godinot, M. F. Seldin, M. G. Caron and B. Giros, *Mol. Pharmacol.*, 1994, **45**, 608.
40. J. A. Morrow, I. T. Collie, D. R. Dunbar, G. B. Walker, M. Shahid and D. R. Hill, *FEBS Lett*, 1998, **439**, 334.
41. K. E. Smith, L. A. Borden, P. R. Hartig, T. Branchek and R. L. Weinshank, *Neuron*, 1992, **8**, 927.
42. Q. R. Liu, B. Lopez-Corcuera, S. Mandiyan, H. Nelson and N. Nelson, *J. Biol. Chem.*, 1993, **268**, 22802.
43. B. Borowsky and B. J. Hoffman, *J. Biol. Chem.*, 1998, **273**, 29077.
44. R. H. Adams, K. Sato, S. Shimada, M. Tohyama, A. W. Puschel and H. Betz, *J. Neurosci.*, 1995, **15**, 2524.
45. F. Zafra and C. Gimenez, *IUBMB Life*, 2008, **60**, 810.
46. B. Cubelos, C. Gimenez and F. Zafra, *Cereb. Cortex*, 2005, **15**, 448.
47. J. Gomeza, S. Hulsman, K. Ohno, V. Eulenburg, K. Szoke, D. Richter and H. Betz, *Neuron*, 2003, **40**, 785.



48. J. Gomez, K. Ohno, S. Hulsman, W. Armsen, V. Eulenburg, D. W. Richter, B. Laube and H. Betz, *Neuron*, 2003, **40**, 797.
49. G. Tsai, R. J. Ralph-Williams, M. Martina, R. Bergeron, J. Berger-Sweeney, K. S. Dunham, Z. Jiang, S. B. Caine and J. T. Coyle, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 8485.
50. B. K. Yee, E. Balic, P. Singer, C. Schwerdel, T. Grampp, L. Gabernet, I. Knuesel, D. Benke, J. Feldon, H. Mohler and D. Boison, *J. Neurosci.*, 2006, **26**, 3169.
51. S. M. Lechner, *Curr. Opin. Pharmacol.*, 2006, **6**, 75.
52. T. M. Bridges, R. Williams and C. W. Lindsley, *Curr. Opin. Mol. Ther.*, 2008, **10**, 591.
53. R. Gilfillan, J. Kerr, G. Walker and G. Wishart, in *Topics in Medicinal Chemistry*, ed. S. Napier and M. Bingham, Springer, Berlin, Heidelberg, 2009, p. 223.
54. K. J. Whitehead, S. M. Pearce, G. Walker, H. Sundaram, D. Hill and N. G. Bowery, *Neuroscience*, 2004, **126**, 381.
55. B. N. Atkinson, S. C. Bell, M. De Vivo, L. R. Kowalski, S. M. Lechner, V. I. Ognyanov, C. S. Tham, C. Tsai, J. Jia, D. Ashton and M. A. Klitenick, *Mol. Pharmacol.*, 2001, **60**, 1414.
56. D. Boulay, P. Pichat, G. Dargazanli, G. Estenne-Bouhtou, J. P. Terranova, N. Rogacki, J. Stemmelin, A. Coste, C. Lanneau, C. Desvignes, C. Cohen, R. Alonso, X. Vige, B. Biton, R. Steinberg, M. Sevrin, F. Oury-Donat, P. George, O. Bergis, G. Griebel, P. Avenet and B. Scatton, *Pharmacol. Biochem. Behav.*, 2008, **91**(1), 47.
57. R. Depoortere, G. Dargazanli, G. Estenne-Bouhtou, A. Coste, C. Lanneau, C. Desvignes, M. Poncelet, M. Heaulme, V. Santucci, M. Decobert, A. Cudennec, C. Voltz, D. Boulay, J. P. Terranova, J. Stemmelin, P. Roger, B. Marabout, M. Sevrin, X. Vige, B. Biton, R. Steinberg, D. Francon, R. Alonso, P. Avenet, F. Oury-Donat, G. Perrault, G. Griebel, P. George, P. Soubrie and B. Scatton, *Neuropsychopharmacology*, 2005, **30**, 1963.
58. M. Martina, Y. Gorfinkel, S. Halman, J. A. Lowe, P. Periyalar, C. J. Schmidt and R. Bergeron, *J. Physiol.*, 2004, **557**, 489.
59. A. Molander, H. H. Lido, E. Lof, M. Ericson and B. Soderpalm, *Alcohol Alcohol.*, 2007, **42**, 11.
60. P. J. Mallorga, J. B. Williams, M. Jacobson, R. Marques, A. Chaudhary, P. J. Conn, D. J. Pettibone and C. Sur, *Neuropharmacology*, 2003, **45**, 585.
61. H. J. Herdon, F. M. Godfrey, A. M. Brown, S. Coulton, J. R. Evans and W. J. Cairns, *Neuropharmacology*, 2001, **41**, 88.
62. J. A. Lowe, III, S. E. Drozda, K. Fisher, C. Strick, L. Lebel, C. Schmidt, D. Hiller and K. S. Zandi, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1291.
63. A. Brown, I. Carlyle, J. Clark, W. Hamilton, S. Gibson, G. McGarry, S. McEachen, D. Rae, S. Thorn and G. Walker, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2007.
64. L. G. Harsing, I. Gacsalyi, G. Szabo, E. Schmidt, N. Sziray, C. Sebban, B. Tesolin-Decros, P. Matyus, A. Egyed, M. Spedding and G. Levay, *Pharmacol. Biochem. Behav.*, 2003, **74**, 811.

65. T. Lipina, V. Labrie, I. Weiner and J. Roder, *Psychopharmacology*, 2005, **179**, 54.
66. K. Kopeck, D. G. Flood, M. Gasior, B. A. McKenna, E. Zuvich, J. Schreiber, J. M. Salvino, J. T. Durkin, M. A. Ator and M. J. Marino, *Biochem. Pharmacol.*, 2010, **80**, 1407.
67. G. Le Pen, J. Kew, D. Alberati, E. Borroni, M. P. Heitz and J. L. Moreau, *Biol. Psychiatry*, 2003, **54**, 1162.
68. D. Manahan-Vaughan, V. Wildforster and C. Thomsen, *Eur. J. Neurosci.*, 2008, **28**, 1342.
69. T. Shimazaki, A. Kaku and S. Chaki, *Psychopharmacology (Berl.)*, 2010, **209**, 263.
70. J. Karasawa, K. Hashimoto and S. Chaki, *Behav. Brain Res.*, 2008, **186**, 78.
71. K. Hashimoto, Y. Fujita, T. Ishima, S. Chaki and M. Iyo, *Eur. Neuro-psychopharmacol.*, 2008, **18**, 414.
72. G. Dargazanli, G. Estenne-Bouhtou, B. Marabout, P. Roger and M. Sevrin, 2004, WO2004013101.
73. S. F. Michardy and Lowe J. A. III, 2006, WO2006106425.
74. J. A. Lowe, III, X. Hou, C. Schmidt, T. F. David, III, S. McHardy, M. Kalman, S. Deninno, M. Sanner, K. Ward, L. Lebel, D. Tunucci, J. Valentine, B. S. Bronk and E. Schaeffer, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2974.
75. S. Jolidon, R. Narquizian, M. Nettekoven, R. Norcross, E. Pinard and H. Stalder, 2005, WO2005014563.
76. E. Pinard, D. Alberati, M. Bender, E. Borroni, V. Brom, S. Burner, H. Fischer, D. Hainzl, R. Halm, N. Hauser, S. Jolidon, J. Lengyel, H. P. Marty, T. Meyer, J. L. Moreau, R. Mory, R. Narquizian, R. D. Norcross, P. Schmid, R. Wermuth and D. Zimmerli, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 6960.
77. D. Boulay, O. Bergis, P. Avenet and G. Griebel, *Neuropsychopharmacology*, 2010, **35**, 416.
78. P. Singer, J. Feldon and B. K. Yee, *Eur. Neuropsychopharmacol.*, 2009, **19**, 571.
79. H. Mohler, U. Rudolph, D. Boison, P. Singer, J. Feldon and B. K. Yee, *Pharmacol. Biochem. Behav.*, 2008, **90**(1), 58.
80. M. Leonetti, C. Desvignes, I. Bougault, J. Souilhac, F. Oury-Donat and R. Steinberg, *Neuroscience*, 2006, **137**, 555.
81. M. D. Black, G. B. Varty, M. Arad, S. Barak, A. De Levie, D. Boulay, P. Pichat, G. Griebel and I. Weiner, *Psychopharmacology (Berl.)*, 2009, **202**, 385.
82. S. Harich, G. Gross and A. Bepalov, *Psychopharmacology (Berl.)*, 2007, **192**, 511.
83. B. M. Roberts, C. L. Shaffer, P. A. Seymour, C. J. Schmidt, G. V. Williams and S. A. Castner, *NeuroReport*, 2010, **21**, 390.
84. T. G. Hamill, W. Eng, A. Jennings, R. Lewis, S. Thomas, S. Wood, L. Street, D. Wisnoski, S. Wolkenberg, C. Lindsley, S. M. Sanabria-Bohorquez,

- S. Patel, K. Riffel, C. Ryan, J. Cook, C. Sur, H. D. Burns and R. Hargreaves, *Synapse*, 2011, **65**, 261.
85. J. Passchier, G. Gentile, R. Porter, H. Herdon, C. Salinas, S. Jakobsen, H. Audrain, M. Laruelle and R. N. Gunn, *Synapse*, 2010, **64**, 542.
86. K. W. Perry, J. F. Falcone, M. J. Fell, J. W. Ryder, H. Yu, P. L. Love, J. Katner, K. D. Gordon, M. R. Wade, T. Man, G. G. Nomikos, L. A. Phebus, A. J. Cauvin, K. W. Johnson, C. K. Jones, B. J. Hoffmann, G. E. Sandusky, M. W. Walter, W. J. Porter, L. Yang, K. M. Merchant, H. E. Shannon and K. A. Svensson, *Neuropharmacology*, 2008, **55**(5), 743.
87. M. Mezler, W. Hornberger, R. Mueller, M. Schmidt, W. Amberg, W. Braje, M. Ochse, H. Schoemaker and B. Behl, *Mol. Pharmacol.*, 2008, **74**, 1705.
88. J. P. Mothet, A. T. Parent, H. Wolosker, R. O. Brady, Jr, D. J. Linden, C. D. Ferris, M. A. Rogawski and S. H. Snyder, *Proc. Natl Acad. Sci. USA*, 2000, **97**, 4926.
89. A. Panatier, D. T. Theodosis, J. P. Mothet, B. Touquet, L. Pollegioni, D. A. Poulain and S. H. Oliet, *Cell*, 2006, **125**, 775.
90. V. Schmieden, J. Kuhse and H. Betz, *Science*, 1993, **262**, 256.
91. G. E. Tsai and P. Y. Lin, *Curr. Pharm. Des.*, 2010, **16**, 522.
92. N. W. Kleckner and R. Dingledine, *Science*, 1988, **241**, 835.
93. T. Matsui, M. Sekiguchi, A. Hashimoto, U. Tomita, T. Nishikawa and K. Wada, *J. Neurochem.*, 1995, **65**, 454.
94. A. J. Berger, S. Dieudonne and P. Ascher, *J. Neurophysiol.*, 1998, **80**, 3336.
95. E. R. Stevens, E. C. Gustafson and R. F. Miller, *Eur. J. Neurosci.*, 2010, **31**, 808.
96. M. J. Schell, M. E. Molliver and S. H. Snyder, *Proc. Natl Acad. Sci. USA*, 1995, **92**, 3948.
97. E. Kartvelishvily, M. Shleper, L. Balan, E. Dumin and H. Wolosker, *J. Biol. Chem.*, 2006, **281**, 14151.
98. H. Wolosker, S. Blackshaw and S. H. Snyder, *Proc. Natl Acad. Sci. USA*, 1999, **96**, 13409.
99. A. Hashimoto, T. Nishikawa, T. Oka and K. Takahashi, *J. Neurochem.*, 1993, **60**, 783.
100. M. J. Schell, R. O. Brady, Jr, M. E. Molliver and S. H. Snyder, *J. Neurosci.*, 1997, **17**, 1604.
101. J. P. Mothet, L. Pollegioni, G. Ouanounou, M. Martineau, P. Fossier and G. Baux, *Proc. Natl Acad. Sci. USA*, 2005, **102**, 5606.
102. E. R. Stevens, M. Esguerra, P. M. Kim, E. A. Newman, S. H. Snyder, K. R. Zahs and R. F. Miller, *Proc. Natl Acad. Sci. USA*, 2003, **100**, 6789.
103. J. P. Mothet, A. T. Parent, H. Wolosker, R. O. Brady, Jr, D. J. Linden, C. D. Ferris, M. A. Rogawski and S. H. Snyder, *Proc. Natl Acad. Sci. USA*, 2000, **97**, 4926.
104. Y. Yang, W. Ge, Y. Chen, Z. Zhang, W. Shen, C. Wu, M. Poo and S. Duan, *Proc. Natl Acad. Sci. USA*, 2003, **100**, 15194.
105. T. E. Salt, *Brain Res.*, 1989, **481**, 403.

106. E. Thiels, D. J. Weisz and T. W. Berger, *Neuroscience*, 1992, **46**, 501.
107. C. Henneberger, T. Papouin, S. H. Oliet and D. A. Rusakov, *Nature*, 2010, **463**, 232.
108. P. Bado, C. Madeira, C. Vargas-Lopes, T. C. Moulin, A. P. Wasilewska-Sampaio, L. Maretti, R. V. de Oliveira, O. B. Amaral and R. Panizzutti, *Psychopharmacology (Berl.)*, 2011, **218**(3), 461.
109. J. D. Vardigan, S. L. Huszar, C. H. McNaughton, P. H. Hutson and J. M. Uslaner, *Pharmacol. Biochem. Behav.*, 2010, **95**, 223.
110. K. Hashimoto, G. Engberg, E. Shimizu, C. Nordin, L. H. Lindstrom and M. Iyo, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2005, **29**, 767.
111. R. Mossner, A. Schuhmacher, M. Wagner, B. B. Quednow, I. Frommann, K. U. Kuhn, S. G. Schwab, M. Rietschel, P. Falkai, W. Wolwer, S. Ruhrmann, A. Bechdorf, W. Gaebel, J. Klosterkotter and W. Maier, *Eur. Arch. Psychiatry Clin. Neurosci.*, 2010, **260**, 209.
112. N. C. Allen, S. Bagade, M. B. McQueen, J. P. Ioannidis, F. K. Kavvoura, M. J. Khoury, R. E. Tanzi and L. Bertram, *Nat. Genet.*, 2008, **40**, 827.
113. P. J. Harrison and D. R. Weinberger, *Mol. Psychiatry*, 2005, **10**, 40.
114. D. J. Muller, C. C. Zai, T. Shinkai, J. Strauss and J. L. Kennedy, *Bipolar. Disord.*, 2011, **13**, 198.
115. H. J. Tuominen, J. Tiihonen and K. Wahlbeck, *Schizophr. Res.*, 2005, **72**, 225.
116. A. R. Rutter, R. L. Fradley, E. M. Garrett, K. L. Chapman, J. M. Lawrence, T. W. Rosahl and S. Patel, *Eur. J. Neurosci.*, 2007, **25**, 1757.
117. X. Xie, T. Dumas, L. Tang, T. Brennan, T. Reeder, W. Thomas, R. D. Klein, J. Flores, B. F. O'Hara, H. C. Heller and P. Franken, *Brain Res.*, 2005, **1052**, 212.
118. J. Nakauchi, H. Matsuo, D. K. Kim, A. Goto, A. Chairoungdua, S. H. Cha, J. Inatomi, Y. Shiokawa, K. Yamaguchi, I. Saito, H. Endou and Y. Kanai, *Neurosci. Lett.*, 2000, **287**, 231.
119. H. Matsuo, Y. Kanai, M. Tokunaga, T. Nakata, A. Chairoungdua, H. Ishimine, S. Tsukada, H. Ooigawa, H. Nawashiro, Y. Kobayashi, J. Fukuda and H. Endou, *Neurosci. Lett.*, 2004, **358**, 123.
120. L. Helboe, J. Egebjerg, M. Moller and C. Thomsen, *Eur. J. Neurosci.*, 2003, **18**, 2227.
121. P. W. Burnet, L. Hutchinson, M. von Hesling, E. J. Gilbert, N. J. Brandon, A. R. Rutter, P. H. Hutson and P. J. Harrison, *Schizophr. Res.*, 2008, **102**, 283.
122. C. Thomsen, L. Helboe and J. Egebjerg, 2003, WO/2003/077998.
123. S. M. Smith, J. M. Uslaner and P. H. Hutson, *Open. Med. Chem. J.*, 2010, **4**, 3.
124. L. Verrall, P. W. Burnet, J. F. Betts and P. J. Harrison, *Mol. Psychiatry*, 2010, **15**, 122.
125. D. V. Ferraris and T. Tsukamoto, *Curr. Pharm. Des.*, 2011, **17**, 103.
126. H. A. Krebs, *Biochem. J.*, 1935, **29**, 1620.
127. L. Pollegioni, L. Piubelli, S. Sacchi, M. S. Pilone and G. Molla, *Cell Mol. Life Sci.*, 2007, **64**, 1373.

128. A. H. Neims, W. D. Zieverink and J. D. Smilack, *J. Neurochem.*, 1966, **13**, 163.
129. K. Momoi, K. Fukui, F. Watanabe and Y. Miyake, *FEBS Lett*, 1988, **238**, 180.
130. L. Verrall, M. Walker, N. Rawlings, I. Benzel, J. N. Kew, P. J. Harrison and P. W. Burnet, *Eur. J. Neurosci.*, 2007, **26**, 1657.
131. S. A. Papagni, A. Mechelli, D. P. Prata, J. Kambeitz, C. H. Fu, M. Picchioni, M. Walshe, T. Touloupoulou, E. Bramon, R. M. Murray, D. A. Collier, A. Bellomo and P. McGuire, *NeuroImage*, 2011, **56**, 2283.
132. P. Roussos, S. G. Giakoumaki, E. Adamaki, G. Anastasios, R. K. Nikos and P. Bitsios, *Neuropsychopharmacology*, 2011, **36**, 1677.
133. C. Madeira, M. E. Freitas, C. Vargas-Lopes, H. Wolosker and R. Panizzutti, *Schizophr. Res.*, 2008, **101**, 76.
134. R. Kapoor, K. S. Lim, A. Cheng, T. Garrick and V. Kapoor, *Brain Res.*, 2006, **1106**, 205.
135. N. C. Stefanis, T. A. Trikalinos, D. Avramopoulos, N. Smyrnis, I. Evdokimidis, E. E. Ntzani, J. P. Ioannidis and C. N. Stefanis, *Biol. Psychiatry*, 2007, **62**, 784.
136. S. L. Christian, J. McDonough, C. Y. Liu, Cy, S. Shaikh, V. Vlamakis, J. A. Badner, A. Chakravarti and E. S. Gershon, *Genomics*, 2002, **79**, 635.
137. T. Shinkai, L. De, V, R. Hwang, D. J. Muller, M. Lanktree, G. Zai, S. Shaikh, G. Wong, T. Sicard, N. Potapova, J. Trakalo, N. King, C. Matsumoto, H. Hori, A. H. Wong, O. Ohmori, F. Macciardi, J. Nakamura and J. L. Kennedy, *NeuroMol. Med.*, 2007, **9**, 169.
138. D. J. Muller, C. C. Zai, T. Shinkai, J. Strauss and J. L. Kennedy, *Bipolar. Disord.*, 2011, **13**, 198.
139. J. Schumacher, R. A. Jamra, J. Freudenberg, T. Becker, S. Ohlraun, A. C. Otte, M. Tullius, S. Kovalenko, A. V. Bogaert, W. Maier, M. Rietschel, P. Propping, M. M. Nothen and S. Cichon, *Mol. Psychiatry*, 2004, **9**, 203.
140. D. M. Otte, A. Bilkei-Gorzo, M. D. Filiou, C. W. Turck, O. Yilmaz, M. I. Holst, K. Schilling, R. Abou-Jamra, J. Schumacher, I. Benzel, W. S. Kunz, H. Beck and A. Zimmer, *Eur. Neuropsychopharmacol.*, 2009, **19**, 339.
141. M. Williams, *Biochem. Pharmacol.*, 2009, **78**, 1360.
142. I. Chumakov, M. Blumenfeld, O. Guerassimenko, L. Cavarec, M. Palicio, H. Abderrahim, L. Bougueleret, C. Barry, H. Tanaka, P. La Rosa, A. Puech, N. Tahri, A. Cohen-Akenine, S. Delabrosse, S. Lissarrague, F. P. Picard, K. Maurice, L. Essieux, P. Millasseau, P. Grel, V. Debailleul, A. M. Simon, D. Caterina, I. Dufaure, K. Malekzadeh, M. Belova, J. J. Luan, M. Bouillot, J. L. Sambucy, G. Primas, M. Saumier, N. Boubkiri, S. Martin-Saumier, M. Nasroune, H. Peixoto, A. Delaye, V. Pinchot, M. Bastucci, S. Guillou, M. Chevillon, R. Sainz-Fuertes, S. Meguenni, J. Aurich-Costa, D. Cherif, A. Gimalac, C. Van Duijn, D. Gauvreau, G. Ouellette, I. Fortier, J. Raelson, T. Sherbatich, N. Riazanskaia, E. Rogaev, P. Raeymaekers, J. Aerssens, F. Konings, W. Luyten, F. Macciardi, P. C. Sham, R. E. Straub, D. R. Weinberger, N. Cohen and D. Cohen, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 13675.

143. S. Sacchi, M. Bernasconi, M. Martineau, J. P. Mothet, M. Ruzzene, M. S. Pilone, L. Pollegioni and G. Molla, *J. Biol. Chem.*, 2008, **283**, 22244.
144. M. Kvajo, A. Dhilla, D. E. Swor, M. Karayiorgou and J. A. Gogos, *Mol. Psychiatry*, 2008, **13**, 685.
145. M. Korostishevsky, M. Kaganovich, A. Cholostoy, M. Ashkenazi, Y. Ratner, D. Dahary, J. Bernstein, U. Bening-Abu-Shach, E. Ben Asher, D. Lancet, M. Ritsner and R. Navon, *Biol. Psychiatry*, 2004, **56**, 169.
146. D. P. Prata, S. A. Papagni, A. Mechelli, C. H. Fu, J. Kambeitz, M. Picchioni, F. Kane, S. Kalidindi, C. McDonald, E. Kravariti, T. Touloupoulou, E. Bramon, M. Walshe, R. Murray, D. A. Collier and P. K. McGuire, *Hum. Brain Mapp.*, 2012, **33**(1), 143.
147. T. E. Goldberg, R. E. Straub, J. H. Callicott, A. Hariri, V. S. Mattay, L. Bigelow, R. Coppola, M. F. Egan and D. R. Weinberger, *Neuropsychopharmacology*, 2006, **31**, 2022.
148. R. Konno and Y. Yasumura, *Genetics*, 1983, **103**, 277.
149. A. Hashimoto, T. Nishikawa, R. Konno, A. Niwa, Y. Yasumura, T. Oka and K. Takahashi, *Neurosci. Lett.*, 1993, **152**, 33.
150. K. Wake, H. Yamazaki, S. Hanzawa, R. Konno, H. Sakio, A. Niwa and Y. Hori, *Neurosci. Lett.*, 2001, **297**, 25.
151. M. Maekawa, M. Watanabe, S. Yamaguchi, R. Konno and Y. Hori, *Neurosci. Res.*, 2005, **53**, 34.
152. S. L. Almond, R. L. Fradley, E. J. Armstrong, R. B. Heavens, A. R. Rutter, R. J. Newman, C. S. Chiu, R. Konno, P. H. Hutson and N. J. Brandon, *Mol. Cell Neurosci.*, 2006, **32**, 324.
153. M. Zhang, M. E. Ballard, A. M. Basso, N. Bratcher, K. E. Browman, P. Curzon, R. Konno, A. H. Meyer and L. E. Rueter, *Behav. Brain Res.*, 2011, **217**, 81.
154. J. N. Kew, A. Koester, J. L. Moreau, F. Jenck, A. M. Ouagazzal, V. Mutel, J. G. Richards, G. Trube, G. Fischer, A. Montkowski, W. Hundt, R. K. Reinscheid, M. Pauly-Evers, J. A. Kemp and H. Bluethmann, *J. Neurosci.*, 2000, **20**, 4037.
155. V. Labrie, W. Wang, S. W. Barger, G. B. Baker and J. C. Roder, *Genes Brain Behav.*, 2010, **9**, 11.
156. V. Labrie, S. Duffy, W. Wang, S. W. Barger, G. B. Baker and J. C. Roder, *Learn. Mem.*, 2009, **16**, 28.
157. V. Labrie, A. H. Wong and J. C. Roder, *Neuropharmacology*, 2012, **62**(3), 1484.
158. V. Labrie, S. J. Clapcote and J. C. Roder, *Pharmacol. Biochem. Behav.*, 2009, **91**, 610.
159. T. Sparey, P. Abeywickrema, S. Almond, N. Brandon, N. Byrne, A. Campbell, P. H. Hutson, M. Jacobson, B. Jones, S. Munshi, D. Pascarella, A. Pike, G. S. Prasad, N. Sachs, M. Sakatis, V. Sardana, S. Venkatraman and M. B. Young, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 3386.
160. D. Ferraris, B. Duvall, Y. S. Ko, A. G. Thomas, C. Rojas, P. Majer, K. Hashimoto and T. Tsukamoto, *J. Med. Chem.*, 2008, **51**, 3357.



161. T. Adage, A. C. Trillat, A. Quattropiani, D. Perrin, L. Cavarec, J. Shaw, O. Guerassimenko, C. Giachetti, B. Greco, I. Chumakov, S. Halazy, A. Roach and P. Zaratini, *Eur. Neuropsychopharmacol.*, 2008, **18**, 200.
162. A. J. Duplantier, S. L. Becker, M. J. Bohanon, K. A. Borzilleri, B. A. Chrnyk, J. T. Downs, L. Y. Hu, A. El-Kattan, L. C. James, S. Liu, J. Lu, N. Maklad, M. N. Mansour, S. Mente, M. A. Piotrowski, S. M. Sakya, S. Sheehan, S. J. Steyn, C. A. Strick, V. A. Williams and L. Zhang, *J. Med. Chem.*, 2009, **52**, 3576.
163. K. Hashimoto, Y. Fujita, M. Horio, S. Kunitachi, M. Iyo, D. Ferraris and T. Tsukamoto, *Biol. Psychiatry*, 2009, **65**, 1103.
164. S. M. Smith, J. M. Uslaner, L. Yao, C. M. Mullins, N. O. Surles, S. L. Huszar, C. H. McNaughton, D. M. Pascarella, M. Kandebo, R. M. Hinchliffe, T. Sparey, N. J. Brandon, B. Jones, S. Venkatraman, M. B. Young, N. Sachs, M. A. Jacobson and P. H. Hutson, *J. Pharmacol. Exp. Ther.*, 2009, **328**, 921.
165. C. E. Ganote, D. R. Peterson and F. A. Carone, *Am. J. Pathol.*, 1974, **77**, 269.
166. M. Orozco-Ibarra, O. N. Medina-Campos, D. J. Sanchez-Gonzalez, C. M. Martinez-Martinez, E. Floriano-Sanchez, A. Santamaria, V. Ramirez, N. A. Bobadilla and J. Pedraza-Chaverri, *Toxicology*, 2007, **229**, 123.
167. C. Hilmas, E. F. Pereira, M. Alkondon, A. Rassoulpour, R. Schwarcz and E. X. Albuquerque, *J. Neurosci.*, 2001, **21**, 7463.
168. T. Ogaya, Z. Song, K. Ishii and T. Fukushima, *Neurochem. Res.*, 2010, **35**, 559.
169. J. Mosbacher, R. Schoepfer, H. Monyer, N. Burnashev, P. H. Seeburg and J. P. Ruppersberg, *Science*, 1994, **266**, 1059.
170. K. M. Partin, D. K. Patneau and M. L. Mayer, *Mol. Pharmacol.*, 1994, **46**, 129.
171. M. D. Black, *Psychopharmacology*, 2005, **179**, 154.
172. A. Copani, A. Genazzani, G. Aleppo, G. Casabona, L. Canonico, U. Scapagnini and F. Nicoletti, *J. Neurochem.*, 1992, **58**, 1199.
173. A. C. Arai, Y. F. Xia, G. Rogers, G. Lynch and M. Kessler, *J. Pharmacol. Exp. Ther.*, 2002, **303**, 1075.
174. G. Rogers and G. Lynch, 1997, WO1997036907.
175. K. A. Yamada and C. M. Tang, *J. Neurosci.*, 1993, **13**, 3904.
176. I. Zivkovic, D. M. Thompson, M. Bertolino, D. Uzunov, M. DiBella, E. Costa and A. Guidotti, *J. Pharmacol. Exp. Ther.*, 1995, **272**, 300.
177. P. Desos, B. Serkiz, P. Morain, J. Lepagnol and A. Cordi, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 3003.
178. P. L. Ornstein, D. M. Zimmerman, M. B. Arnold, T. J. Bleisch, B. Cantrell, R. Simon, H. Zarrinmayeh, S. R. Baker, M. Gates, J. P. Tizzano, D. Bleakman, A. Mandelzys, K. R. Jarvie, K. Ho, M. Deverill and R. K. Kamboj, *J. Med. Chem.*, 2000, **43**, 4354.
179. M. B. Arnold, S. R. Baker, D. Bleakman, T. J. Bleisch, B. Cantrell, A. Escribano, K. Matsumoyo, T. McKennon, P. L. Ornstein, R. L. Simon,

- E. C. Smith, J. P. Tizzano, H. Zarrinmayeh and D. M. Zimmerman, 1998, WO1998033496.
180. D. M. Bradley, W. N. Chan, S. Harrison, R. Thatcher, K. M. Thewlis and S. E. Ward, 2007, WO2007107539.
  181. J. Larson, C. N. Quach, B. Q. LeDuc, A. Nguyen, G. A. Rogers and G. Lynch, *Brain Res.*, 1996, **738**, 353.
  182. L. C. Palmer, U. S. Hess, J. Larson, G. A. Rogers, C. M. Gall and G. Lynch, *Brain Res. Mol. Brain Res.*, 1997, **46**, 127.
  183. K. E. Vanover, *Eur. J. Pharmacol.*, 1997, **332**, 115.
  184. S. A. Johnson, N. T. Luu, T. A. Herbst, R. Knapp, D. Lutz, A. Arai, G. A. Rogers and G. Lynch, *J. Pharmacol. Exp. Ther.*, 1999, **289**, 392.
  185. C. K. Olsen, M. Kreilgaard and M. Didriksen, *Pharmacol. Biochem. Behav.*, 2006, **84**, 259.
  186. U. S. Hess, S. P. Whalen, L. M. Sandoval, G. Lynch and C. M. Gall, *Neuroscience*, 2003, **121**, 509.
  187. G. Lynch, *Curr. Opin. Pharmacol.*, 2004, **4**, 4.
  188. G. Lynch, *Curr. Opin. Pharmacol.*, 2006, **6**, 82.
  189. M. J. O'Neill, D. Bleakman, D. M. Zimmerman and E. S. Nisenbaum, *Curr. Drug. Targets CNS Neurol. Disord*, 2004, **3**, 181.
  190. M. J. O'Neill and S. Dix, *IDrugs*, 2007, **10**, 185.
  191. J. J. Hagan and D. N. C. Jones, *Schizophr. Bull.*, 2005, **31**, 830.
  192. Z. Abdul-Monim, G. P. Reynolds and J. C. Neill, *Behav. Brain Res.*, 2006, **169**, 263.
  193. S. L. McLean, J. P. Beck, M. L. Woolley and J. C. Neill, *Behav. Brain Res.*, 2008, **189**, 152.
  194. B. Grayson, N. F. Idris and J. C. Neill, *Behav. Brain Res.*, 2007, **184**, 31.
  195. T. Damgaard, D. B. Larsen, S. L. Hansen, B. Grayson, J. C. Neill and N. Plath, *Behav. Brain Res.*, 2010, in press, corrected proof.
  196. M. J. O'Neill and J. M. Witkin, *Curr. Drug Targets*, 2007, **8**, 603.
  197. N. Jones, M. J. Messenger, M. J. O'Neill, A. Oldershaw, G. Gilmour, R. M. A. Simmons, S. Iyengar, V. Libri, M. Tricklebank and S. C. R. Williams, *Neuropsychopharmacology*, 2007, **33**, 1713.
  198. J. Ren, B. Y. Poon, Y. Tang, G. D. Funk and J. J. Greer, *Am. J. Respir. Crit. Care Med.*, 2006, **174**, 1384.
  199. S. Marengo and D. R. Weinberger, *CNS Drugs*, 2006, **20**, 173.
  200. J. Zarate and H. K. Manji, *Exp. Neurol.*, 2008, **211**, 7.
  201. G. Lynch, R. Granger, J. Ambros-Ingerson, C. M. Davis, M. Kessler and R. Schehr, *Exp. Neurol.*, 1997, **145**, 89.
  202. G. Lynch, M. Kessler, G. Rogers, J. Ambros-Ingerson, R. Granger and R. S. Schehr, *Int. Clin. Psychopharmacol.*, 1996, **11**, 13.
  203. M. Ingvar, J. Ambros-Ingerson, M. Davis, R. Granger, M. Kessler, G. A. Rogers, R. S. Schehr and G. Lynch, *Exp. Neurol.*, 1997, **146**, 553.
  204. S. Marengo, M. F. Egan, T. E. Goldberg, M. B. Knable, R. K. McClure, G. Winterer and D. R. Weinberger, *Schizophr. Res*, 2002, **57**, 221.
  205. D. C. Goff, L. Leahy, I. Berman, T. Posever, L. Herz, A. C. Leon, S. A. Johnson and G. Lynch, *J. Clin. Psychopharmacol.*, 2001, **21**, 484.



206. D. C. Goff, J. S. Lamberti, A. C. Leon, M. F. Green, A. L. Miller, J. Patel, T. Manschreck, O. Freudenreich and S. A. Johnson, *Neuropsychopharmacology*, 2008, **33**, 465.
207. E. Wezenberg, R. J. Verkes, G. S. Ruigt, W. Hulstijn and B. G. Sabbe, *Neuropsychopharmacology*, 2007, **32**, 1272.
208. L. J. Porrino, J. B. Daunais, G. A. Rogers, R. E. Hampson and S. A. Deadwyler, *PLoS Biol.*, 2005, **3**, e299.
209. N. J. Wassensten, R. M. Reichardt and T. J. Balkin, *Aviat. Space Environ. Med.*, 2007, **78**, 937.
210. A. S. Chappell, C. Gonzales, J. Williams, M. M. Witte, R. C. Mohs, R. Sperling, A. S. Chappell, C. Gonzales, J. Williams, M. M. Witte, R. C. Mohs and R. Sperling, *Neurology*, 2007, **68**, 1008.
211. R. A. Bressan and L. S. Pilowsky, *Eur. J. Nucl. Med.*, 2000, **27**, 1723.
212. N. Jones, M. J. O'Neill, M. Tricklebank, V. Libri, S. C. Williams, N. Jones, M. J. O'Neill, M. Tricklebank, V. Libri and S. C. R. Williams, *Psychopharmacology*, 2005, **180**, 743.
213. F. Nicoletti, J. Bockaert, G. L. Collingridge, P. J. Conn, F. Ferraguti, D. D. Schoepp, J. T. Wroblewski and J. P. Pin, *Neuropharmacology*, 2011, **60**, 1017.
214. J. P. Pin and R. Duvoisin, *Neuropharmacology*, 1995, **34**, 1.
215. M. J. Fell, D. L. McKinzie, J. A. Monn and K. A. Svensson, *Neuropharmacology*, 2012, **62**(3), 1473.
216. G. J. Marek, *Eur. J. Pharmacol.*, 2010, **639**, 81.
217. P. N. Vinson and P. J. Conn, *Neuropharmacology*, 2012, **62**(3), 1461.
218. S. B. Marti, S. Cichon, P. Propping and M. Nothen, *Am. J. Med. Genet.*, 2002, **114**, 12.
219. S. W. Scherer, R. M. Duvoisin, R. Kuhn, H. H. Heng, E. Belloni and L. C. Tsui, *Genomics*, 1996, **31**, 230.
220. M. F. Egan, R. E. Straub, T. E. Goldberg, I. Yakub, J. H. Callicott, A. R. Hariri, V. S. Mattay, A. Bertolino, T. M. Hyde, C. Shannon-Weickert, M. Akil, J. Crook, R. K. Vakkalanka, R. Balkissoon, R. A. Gibbs, J. E. Kleinman and D. R. Weinberger, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 12604.
221. R. Mossner, A. Schuhmacher, S. Schulze-Rauschenbach, K. U. Kuhn, D. Rujescu, M. Rietschel, A. Zobel, P. Franke, W. Wolwer, W. Gaebel, H. Hafner, M. Wagner and W. Maier, *Eur. Neuropsychopharmacol.*, 2008, **18**, 768.
222. B. T. Baune, T. Suslow, C. Beste, E. Birosova, K. Domschke, C. Schlmeyer and C. Konrad, *Genes Brain Behav.*, 2010, **9**, 459.
223. J. R. Bishop, D. D. Miller, V. L. Ellingrod and T. Holman, *Hum. Psychopharmacol.*, 2011, **26**(1), 28.
224. S. B. Marti, S. Cichon, P. Propping and M. Nothen, *Am. J. Med. Genet.*, 2002, **114**, 46.
225. N. Norton, H. J. Williams, S. Dwyer, D. Ivanov, A. C. Preece, A. Gerrish, N. M. Williams, P. Yerassimou, S. Zammit, M. C. O'Donovan and M. J. Owen, *BMC Psychiatry*, 2005, **5**, 23.

226. S. Ghose, K. A. Gleason, B. W. Potts, K. Lewis-Amezcuca and C. A. Tamminga, *Am. J. Psychiatry*, 2009, **166**, 812.
227. C. Corti, L. Crepaldi, S. Mion, A. L. Roth, J. H. Xuereb and F. Ferraguti, *Biol. Psychiatry*, 2007, **62**, 747.
228. A. Joo, H. Shibata, H. Ninomiya, H. Kawasaki, N. Tashiro and Y. Fukumaki, *Mol. Psychiatry*, 2001, **6**, 186.
229. T. Tsunoka, T. Kishi, T. Kitajima, T. Okochi, T. Okumura, Y. Yamanouchi, Y. Kinoshita, K. Kawashima, H. Naitoh, T. Inada, H. Ujike, M. Yamada, N. Uchimura, I. Sora, M. Iyo, N. Ozaki and N. Iwata, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2010, **34**, 639.
230. J. Gonzalez-Maesó, R. L. Ang, T. Yuen, P. Chan, N. V. Weisstaub, J. F. Lopez-Gimenez, M. Zhou, Y. Okawa, L. F. Callado, G. Milligan, J. A. Gingrich, M. Filizola, J. J. Meana and S. C. Sealson, *Nature*, 2008, **452**, 93.
231. G. Gu, D. S. Lorrain, H. Wei, R. L. Cole, X. Zhang, L. P. Daggett, H. J. Schaffhauser, L. J. Bristow and S. M. Lechner, *Brain Res.*, 2008, **1197**, 47.
232. M. Julio-Pieper, P. J. Flor, T. G. Dinan and J. F. Cryan, *Pharmacol. Rev.*, 2011, **63**, 35.
233. J. A. Monn, M. J. Valli, S. M. Massey, R. A. Wright, C. R. Salhoff, B. G. Johnson, T. Howe, C. A. Alt, G. A. Rhodes, R. L. Robey, K. R. Griffey, J. P. Tizzano, M. J. Kallman, D. R. Helton and D. D. Schoepp, 1997, **40**, 528.
234. J. A. Monn, S. M. Massey, M. J. Valli, S. S. Henry, G. A. Stephenson, M. Bures, M. Herin, J. Catlow, D. Giera, R. A. Wright, B. G. Johnson, S. L. Andis, A. Kingston and D. D. Schoepp, *J. Med. Chem.*, 2007, **50**, 233.
235. B. Moghaddam and B. W. Adams, *Science*, 1998, **281**, 1349.
236. S. T. Patil, L. Zhang, F. Martenyi, S. L. Lowe, K. A. Jackson, B. V. Andreev, A. S. Avedisova, L. M. Bardenstein, I. Y. Gurovich, M. A. Morozova, S. N. Mosolov, N. G. Neznanov, A. M. Reznik, A. B. Smulevich, V. A. Tochilov, B. G. Johnson, J. A. Monn and D. D. Schoepp, *Nat. Med.*, 2007, **13**, 1102.
237. B. J. Kinon, L. Zhang, B. A. Millen, O. O. Osuntokun, J. E. Williams, S. Kollack-Walker, K. Jackson, L. Kryzhanovskaya and N. Jarkova, *J. Clin. Psychopharmacol.*, 2011, **31**, 349.
238. E. Dunayevich, J. Erickson, L. Levine, R. Landbloom, D. D. Schoepp and G. D. Tollefson, *Neuropsychopharmacology*, 2008, **33**, 1603.
239. M. J. Fell, K. A. Svensson, B. G. Johnson and D. D. Schoepp, *J. Pharmacol. Exp. Ther.*, 2008, **326**, 209.
240. R. Galici, C. K. Jones, K. Hemstapat, Y. Nong, N. G. Echemendia, L. C. Williams, T. de Paulis and P. J. Conn, *J. Pharmacol. Exp. Ther.*, 2006, **318**, 173.
241. C. Bonnefous, J. M. Vernier, J. H. Hutchinson, M. F. Gardner, M. Cramer, J. K. James, B. A. Rowe, L. P. Daggett, H. Schaffhauser and T. M. Kamenecka, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4354.
242. M. P. Johnson, M. Baez, G. E. Jagdmann, Jr, T. C. Britton, T. H. Large, D. O. Callagaro, J. P. Tizzano, J. A. Monn and D. D. Schoepp, *J. Med. Chem.*, 2003, **46**, 3189.

243. B. A. Rowe, H. Schaffhauser, S. Morales, L. S. Lubbers, C. Bonnefous, T. M. Kamenecka, J. McQuiston and L. P. Daggett, *J. Pharmacol. Exp. Ther.*, 2008, **326**, 240.
244. K. Hemstapat, H. Da Costa, Y. Nong, A. E. Brady, Q. Luo, C. M. Niswender, G. D. Tamagnan and P. J. Conn, *J. Pharmacol. Exp. Ther.*, 2007, **322**, 254.
245. T. D. Aicher, D. R. Benesh, M.-J. Blanco-Pillardo, G. Cortez, T. D. Groendyke, Khilevich, A., J. A. Knobelsdorf, F. P. Marmsater J. M. Schkeryantz and T. P. Tang, 2006, WO2006057860.
246. J. M. Cid-Nunez, A. A. Trabanco-Suarez, G. J. Macdonald and G. A. J. Duvey, 2008, WO2008107481.
247. S. Clayton, F. Ma, B. Van Wagenen, R. Ukkiramapandian, I. Egle, J. Empfield, M. Isaac, A. Slassi, G. Steelman, R. Urbanek and S. Walsh, 2006, WO2006020879.
248. P. L. D'Alessandro, C. Corti, A. Roth, A. Ugolini, A. Sava, D. Montanari, F. Bianchi, S. L. Garland, B. Powney, E. L. Koppe, M. Rocheville, G. Osborne, P. Perez, F. J. de la, F. M. De Los, P. W. Smith, C. Branch, D. Nash and S. P. Watson, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 759.
249. E. J. Brnardic, M. E. Fraley, R. M. Garbaccio, M. E. Layton, J. M. Sanders, C. Culberson, M. A. Jacobson, B. C. Magliaro, P. H. Hutson, J. A. O'Brien, S. L. Huszar, J. M. Uslaner, K. L. Fillgrove, C. Tang, Y. Kuo, S. M. Sur and G. D. Hartman, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3129.
250. M. A. Benneyworth, Z. Xiang, R. L. Smith, E. E. Garcia, P. J. Conn and E. Sanders-Bush, *Mol. Pharmacol.*, 2007, **72**, 477.
251. E. A. Hackler, N. E. Byun, C. K. Jones, J. M. Williams, R. Baheza, S. Sengupta, M. D. Grier, M. Avison, P. J. Conn and J. C. Gore, *Neuroscience*, 2010, **168**, 209.
252. R. Galici, N. G. Echemendia, A. L. Rodriguez and P. J. Conn, *J. Pharmacol. Exp. Ther.*, 2005, **315**, 1181.
253. A. Nikiforuk, P. Popik, K. U. Drescher, M. van Gaalen, A. L. Relo, M. Mezler, G. Marek, H. Schoemaker, G. Gross and A. Bepalov, *J. Pharmacol. Exp. Ther.*, 2010, **335**, 665.
254. D. Xi, Y. C. Li, M. A. Snyder, R. Y. Gao, A. E. Adelman, W. Zhang, J. S. Shumsky and W. J. Gao, *Neuropsychopharmacology*, 2011, **36**, 1260.
255. C. F. Fuente-Sandoval, P. F. Leon-Ortiz, R. F. Favila, S. Stephano, D. Mamo, J. F. Ramirez-Bermudez and A. Graff-Guerrero, *Neuropsychopharmacology*, 2011, **36**, 1781.
256. R. Anwyl, *Brain Res. Brain Res. Rev.*, 1999, **29**, 83.
257. G. K. Aghajanian and G. J. Marek, *Brain Res. Brain Res. Rev.*, 2000, **31**, 302.
258. B. Moghaddam and B.W. Adams, *Science*, 1998, **281**, 1349.
259. G. Molinaro, A. Traficante, B. Riozzi, L. Di Menna, M. Curto, S. Pallottino, F. Nicoletti, V. Bruno and G. Battaglia, *Mol. Pharmacol.*, 2009, **76**, 379.
260. G. J. Marek, R. A. Wright, D. D. Schoepp, J. A. Monn and G. K. Aghajanian, *J. Pharmacol. Exp. Ther.*, 2000, **292**, 76.

261. J. C. Gewirtz and G. J. Marek, *Neuropsychopharmacology*, 2000, **23**, 569.
262. J. L. Moreno, T. Holloway, L. Albizu, S. C. Sealfon and J. Gonzalez-Maeso, *Neurosci. Lett.*, 2011, **493**, 76.
263. W. Liu, A. C. Downing, L. M. Munsie, P. Chen, M. R. Reed, C. L. Ruble, K. T. Landschulz, B. J. Kinon and L. K. Nisenbaum, *Pharmacogenomics J.*, 2012, **12**(3), 246.
264. K. R. Hoyt, S. Rajdev, C. L. Fattman and I. J. Reynolds, *J. Neurochem.*, 1995, **64**, 2049.
265. R. S. Devon, S. Anderson, P. W. Teague, W. J. Muir, V. Murray, A. J. Pelosi, D. H. Blackwood and D. J. Porteous, *Mol. Psychiatry*, 2001, **6**, 311.
266. L. P. Daggett, A. I. Sacaan, M. Akong, S. P. Rao, S. D. Hess, C. Liaw, A. Urrutia, C. Jachec, S. B. Ellis and J. Dreessen, *Neuropharmacology*, 1995, **34**, 871.
267. R. Shigemoto, S. Nomura, H. Ohishi, H. Sugihara, S. Nakanishi and N. Mizuno, *Neurosci. Lett.*, 1993, **163**, 53.
268. C. Romano, M. A. Sesma, C. T. McDonald, K. O'Malley, A. N. van den Pol and J. W. Olney, *J. Comp. Neurol.*, 1995, **355**, 455.
269. R. Lujan, Z. Nusser, J. D. Roberts, R. Shigemoto and P. Somogyi, *Eur. J. Neurosci.*, 1996, **8**, 1488.
270. V. Musante, E. Neri, M. Feligioni, A. Puliti, M. Pedrazzi, V. Conti, C. Usai, A. Diaspro, R. Ravazzolo, J. M. Henley, G. Battaglia and A. Pittaluga, *Neuropharmacology*, 2008, **55**, 474.
271. K. Blondeau, *Neurogastroenterol. Motil.*, 2010, **22**, 836.
272. A. Satow, S. Maehara, S. Ise, H. Hikichi, M. Fukushima, G. Suzuki, T. Kimura, T. Tanak, S. Ito, H. Kawamoto and H. Ohta, *J. Pharmacol. Exp. Ther.*, 2008, **326**, 577.
273. A. Lesage and T. Steckler, *Eur. J. Pharmacol.*, 2010, **639**, 2.
274. A. Satow, G. Suzuki, S. Maehara, H. Hikichi, T. Murai, H. Kawagoe-Takaki, M. Hata, S. Ito, S. Ozaki, H. Kawamoto and H. Ohta, *J. Pharmacol. Exp. Ther.*, 2009, **330**, 179.
275. T. Ohnuma, S. Tessler, H. Arai, R. L. Faull, P. J. McKenna and P. C. Emson, *Brain Res. Mol. Brain Res.*, 2000, **85**, 24.
276. T. Ohnuma, S. J. Augood, H. Arai, P. J. McKenna and P. C. Emson, *Brain Res. Mol. Brain Res.*, 1998, **56**, 207.
277. D. D. Schoepp, J. Goldsworthy, B. G. Johnson, C. R. Salhoff and S. R. Baker, *J. Neurochem.*, 1994, **63**, 769.
278. A. J. Doherty, M. J. Palmer, J. M. Henley, G. L. Collingridge and D. E. Jane, *Neuropharmacology*, 1997, **36**, 265.
279. G. Mannaioni, S. Attucci, A. Missanelli, R. Pellicciari, R. Corradetti and F. Moroni, *Neuropharmacology*, 1999, **38**, 917.
280. F. Gasparini, K. Lingenhohl, N. Stoehr, P. J. Flor, M. Heinrich, I. Vranesic, M. Biollaz, H. Allgeier, R. Heckendorn, S. Urwyler, M. A. Varney, E. C. Johnson, S. D. Hess, S. P. Rao, A. I. Sacaan, E. M. Santori, G. Velicelebi and R. Kuhn, *Neuropharmacology*, 1999, **38**, 1493.

281. J. J. Anderson, S. P. Rao, B. Rowe, D. R. Giracello, G. Holtz, D. F. Chapman, L. Tehrani, M. J. Bradbury, N. D. Cosford and M. A. Varney, *J. Pharmacol. Exp. Ther.*, 2002, **303**, 1044.
282. P. Malherbe, N. Kratochwil, A. Muhlemann, M. T. Zenner, C. Fischer, M. Stahl, P. R. Gerber, G. Jaeschke and R. H. Porter, *J. Neurochem.*, 2006, **98**, 601.
283. P. Malherbe, N. Kratochwil, M. T. Zenner, J. Piussi, C. Diener, C. Kratzeisen, C. Fischer and R. H. Porter, *Mol. Pharmacol.*, 2003, **64**, 823.
284. J. A. O'Brien, W. Lemaire, T. B. Chen, R. S. Chang, M. A. Jacobson, S. N. Ha, C. W. Lindsley, H. J. Schaffhauser, C. Sur, D. J. Pettibone, P. J. Conn and D. L. Williams, Jr, *Mol. Pharmacol.*, 2003, **64**, 731.
285. C. W. Lindsley, D. D. Wisnoski, W. H. Leister, J. O'Brien, W. Lemaire, D. L. Williams, Jr, M. Burno, C. Sur, G. G. Kinney, D. J. Pettibone, P. R. Tiller, S. Smith, M. E. Duggan, G. D. Hartman, P. J. Conn and J. R. Huff, *J. Med. Chem.*, 2004, **47**, 5825.
286. J. A. O'Brien, W. Lemaire, M. Wittmann, M. A. Jacobson, S. N. Ha, D. D. Wisnoski, C. W. Lindsley, H. J. Schaffhauser, B. Rowe, C. Sur, M. E. Duggan, D. J. Pettibone, P. J. Conn and D. L. Williams, Jr, *J. Pharmacol. Exp. Ther.*, 2004, **309**, 568.
287. F. Liu, S. Grauer, C. Kelley, R. Navarra, R. Graf, G. Zhang, P. J. Atkinson, M. Popiolek, C. Wantuch, X. Khawaja, D. Smith, M. Olsen, E. Kouranova, M. Lai, F. Pruthi, C. Pulicicchio, M. Day, A. Gilbert, M. H. Pausch, N. J. Brandon, C. E. Beyer, T. A. Comery, S. Logue, S. Rosenzweig-Lipson and K. L. Marquis, *J. Pharmacol. Exp. Ther.*, 2008, **327**, 827.
288. A. L. Rodriguez, M. D. Grier, C. K. Jones, E. J. Herman, A. S. Kane, R. L. Smith, R. Williams, Y. Zhou, J. E. Marlo, E. L. Days, T. N. Blatt, S. Jadhav, U. N. Menon, P. N. Vinson, J. M. Rook, S. R. Stauffer, C. M. Niswender, C. W. Lindsley, C. D. Weaver and P. J. Conn, *Mol. Pharmacol.*, 2010, **78**, 1105.
289. N. Spear, R. A. Gadiant, D. E. Wilkins, M. Do, J. S. Smith, K. L. Zeller, P. Schroeder, M. Zhang, J. Arora and V. Chhajlani, *Eur. J. Pharmacol.*, 2011, **659**, 146.
290. A. S. Hammond, A. L. Rodriguez, S. D. Townsend, C. M. Niswender, K. J. Gregory, C. W. Lindsley and P. J. Conn, *ACS Chem. Neurosci.*, 2010, **1**, 702.
291. Y. Chen, C. Goudet, J. P. Pin and P. J. Conn, *Mol. Pharmacol.*, 2008, **73**, 909.
292. S. J. Bradley, C. J. Langmead, J. M. Watson and R. A. Challiss, *Mol. Pharmacol.*, 2011, **79**, 874.
293. R. Anwyl, *Neuropharmacology*, 2009, **56**, 735.
294. J. E. Ayala, Y. Chen, J. L. Banko, D. J. Sheffler, R. Williams, A. N. Telk, N. L. Watson, Z. Xiang, Y. Zhang, P. J. Jones, C. W. Lindsley, M. F. Olive and P. J. Conn, *Neuropsychopharmacology*, 2009, **34**, 2057.
295. K. S. Kroker, G. Rast and H. Rosenbrock, *Neuropharmacology*, 2011, **61**, 707.

296. D. Balschun, W. Zusratter and W. Wetzl, *Neuroscience*, 2006, **142**, 691.
297. K. Vales, J. Svoboda, K. Benkovicova, V. Bubenikova-Valesova and A. Stuchlik, *Eur. J. Pharmacol.*, 2010, **639**, 91.
298. J. M. Uslaner, S. Parmentier-Batteur, R. B. Flick, N. O. Surles, J. S. Lam, C. H. McNaughton, M. A. Jacobson and P. H. Hutson, *Neuropharmacology*, 2009, **57**, 531.
299. A. P. Yiu, A. J. Rashid and S. A. Josselyn, *Neuropsychopharmacology*, 2011, **36**, 2169.
300. A. Barco, C. Pittenger and E. R. Kandel, *Expert Opin. Ther. Targets*, 2003, **7**, 101.
301. J. M. Darrah, M. R. Stefani and B. Moghaddam, *Behav. Pharmacol.*, 2008, **19**, 225.
302. G. G. Kinney, M. Burno, U. C. Campbell, L. M. Hernandez, D. Rodriguez, L. J. Bristow and P. J. Conn, *J. Pharmacol. Exp. Ther.*, 2003, **306**, 116.
303. S. A. Brody, F. Conquet and M. A. Geyer, *Psychopharmacology (Berl.)*, 2004, **172**, 187.
304. S. Parmentier-Batteur, J. A. O'Brien, S. Doran, S. J. Nguyen, R. B. Flick, J. M. Uslaner, H. Chen, E. N. Finger, T. M. Williams, M. A. Jacobson and P. H. Hutson, *Neuropharmacology*, 2012, **62**(3), 1453.
305. J. Lowe, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1291.
306. S. G. Gibson, D. R. Jaap, S.N. Thorn and R. Gilfillan, WO2000007978, 2000.

## CHAPTER 5

# *Discovery and Clinical Data for a Novel AMPA Receptor Positive Modulator*

SIMON E. WARD

Professor of Medicinal Chemistry & Director of Translational Drug  
Discovery Group, School of Life Sciences, University of Sussex, Brighton,  
BN1 9QJ, United Kingdom  
Email: [simon.ward@sussex.ac.uk](mailto:simon.ward@sussex.ac.uk)

## 5.1 Introduction

This chapter describes part of a research project which ran over several years to identify novel AMPA receptor positive allosteric modulators as agents to enhance the cognitive deficits of schizophrenia.

Schizophrenia is a severe, debilitating disorder that is manifest as multiple symptoms, including hallucinations, disordered thinking, delusions (positive symptoms), anhedonia, blunted affect, social inadequacy (negative symptoms) and impairment of executive functions, memory and attention (cognitive symptoms). It affects ~1–1.3% of the adult population and most commonly appears in early adulthood and persists throughout life.

Hyperactivity of the mesolimbic dopaminergic pathway has been suggested to cause the positive symptoms of schizophrenia whereas hypoactivity of the mesocortical dopaminergic pathway is believed to contribute to the negative and cognitive symptoms of the disease. As such, dopaminergic partial agonists/antagonists are the front-line treatment for schizophrenia. Alternatively, dysfunctional corticolimbic glutamatergic neurotransmission (hypoglutamatergia)

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)



has been implicated in the pathophysiology of schizophrenia. Glutamatergic neurons represent the primary excitatory afferent and efferent systems innervating the cortex, limbic regions and striatum. Glutamatergic dysfunction may be particularly relevant to those forms of schizophrenia in which negative symptoms, cognitive deficits and deterioration are prominent features.<sup>1</sup>

More recently, however, there has been some reconciliation between hyperdopaminergic and hypoglutamatergic theories of schizophrenia suggesting that interconnected abnormalities of both systems occur.<sup>2</sup> In this regard, imaging data are consistent with the idea that dopaminergic dysregulation might be secondary to synaptic disconnectivity in the prefrontal cortex, which can be modelled by glutamate receptor antagonists. A deficit in glutamatergic neurotransmission could lead to excessive subcortical dopaminergic activity, which in turn could exacerbate the glutamate deficit further leading to a reduction in neuronal connectivity and plasticity.

Historically, patients were treated with so-called typical antipsychotic drugs (*e.g.* chlorpromazine, haloperidol and thioridazine also termed neuroleptics), although these agents have now been largely superseded by the introduction of atypical agents *e.g.* clozapine, olanzapine, risperidone, ziprasidone and aripiprazole. Both classes of drug antagonize dopamine D<sub>2/3</sub> receptors and thereby ameliorate the positive symptoms of the disease. However, typical antipsychotic drugs commonly induce “Parkinsonian” extra-pyramidal side-effects whereas atypical antipsychotic drugs do not, principally, it is thought, because they also antagonize 5-HT<sub>2</sub> receptors, although this is not the only explanation. Despite their superior side-effect profile atypical antipsychotic agents still induce a considerable number of side-effects (including weight gain and hyperprolactinaemia) and are still relatively weak at ameliorating both the negative and cognitive symptoms of the disease.<sup>3</sup>

There is a wealth of evidence in the literature identifying cognition as a core symptom of schizophrenia and highlighting the limitations of current antipsychotics in treating these symptoms (see review 4). In comparison with healthy controls, 98% of schizophrenic patients exhibit cognitive performance below expected levels. Cognitive impairment, in contrast to positive and negative symptoms, appears to be evident as a risk factor in advance of psychosis, adversely affects social interaction and routine daily activity and is therefore related to a poor functional outcome. The extent of cognitive impairments in schizophrenia is wide ranging, including recognition memory, attention, reasoning, problem solving and verbal memory and in longitudinal studies appears to be relatively stable from the first onset through to middle age. Pharmacological treatment of cognitive impairment should address the aims of improving daily functional activity and social interaction and lead to a reduction in long-term morbidity. As such, it is essential to develop therapeutic agents that address the medical need to treat cognitive dysfunction in schizophrenia, not only as an add-on therapy to established antipsychotic agents but also because there is emerging recognition that selective treatment of this symptom domain will translate to improved functional outcome for the patient. This has been widely recognized, as evidenced by the TURNs (Treatment Units for Research



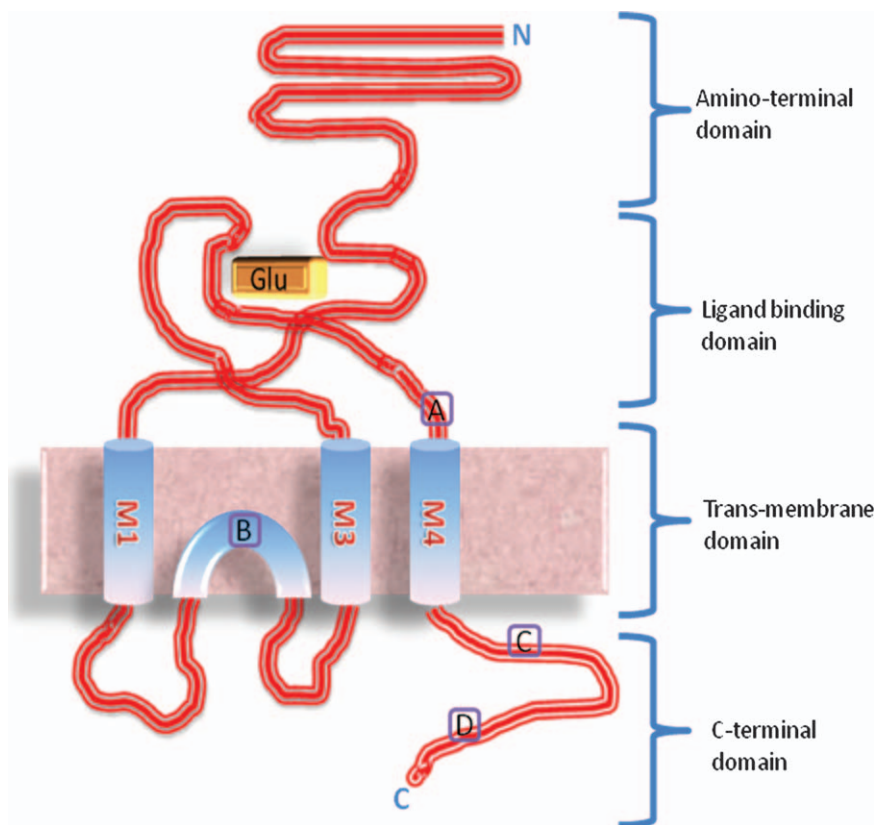
on Neurocognition and Schizophrenia) initiative.<sup>5</sup> This programme, funded by the National Institute of Mental Health, initially selected an AMPA receptor (AMPA) positive modulator Farampator (CX691, ORG24448) to conduct a proof of concept clinical study for the treatment of cognitive deficits in schizophrenia, although the study was subsequently terminated pending the outcome of a cardiac safety study in patients. AMPAR positive modulators have been shown to improve performance in a range of preclinical models and mediate their cognition-enhancing effect by potentiating glutamatergic synaptic neurotransmission.

### **AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) Receptors**

Given the evidence that a hypoglutamatergic state underlies many of the symptoms of schizophrenia, enhancing AMPAR function has been proposed as a promising approach for treating this disorder. Targeting of specific AMPARs is complicated by the fact that this ionotropic receptor consists of a family of hetero-oligomeric (tetrameric) receptors arising from four genes, each of which encodes a distinct receptor subunit (termed GluR1-4 or GluRA-D and more recently Glu(A1) – Glu(A4)).<sup>6</sup> Each subunit contains approximately 900 amino acids and exhibits around 65–75% sequence homology to other subunits. Each subunit is glycosylated and possesses a long extra-cellular amino-terminus, a short intra-cellular carboxy-terminus and has one intra-membrane (M2) as well as three membrane-spanning (M1, M3 and M4) hydrophobic domains (see Figure 5.1). Furthermore, Q/R editing in TM2 of the GluA2 subunit controls the  $\text{Ca}^{2+}$  permeability of this receptor, with AMPARs in mature brain generally containing a GluA2 subunit with an R residue in TM2 which restricts  $\text{Ca}^{2+}$  flux through the channel and essentially renders the receptor permeable to just  $\text{Na}^{+}$  and  $\text{K}^{+}$ .<sup>6,7</sup>

Recombinant homotetrameric AMPARs, comprising four identical subunits, are functionally active and can be used in various *in vitro* assays. However, the functional and anatomical diversity of AMPARs throughout the CNS is manifest by the different possible subunit permutations. AMPAR subunit stoichiometry influences the biophysical and functional properties of the receptor by modifying parameters such as receptor kinetics, channel open time, internalization and trafficking to, and from, the post-synaptic membrane.

Although evidence from the literature suggests that AMPARs are widely expressed in the periphery of lower species (rat), less is known about their distribution in higher species, including man. AMPARs are most highly expressed in the brain and are responsible for the majority of fast glutamatergic synaptic transmission. In this respect, during periods of repetitive glutamatergic afferent input AMPAR-mediated synaptic transmission activates the *N*-methyl *D*-aspartate (NMDA) subtype of glutamate receptor (by relieving the voltage-dependent  $\text{Mg}^{2+}$  block of its channel) so that it can contribute to fast synaptic transmission and, by enabling  $\text{Ca}^{2+}$  influx into the post-synaptic neurone, induce forms of synaptic plasticity (*e.g.* long-term potentiation, LTP, and long-term depression, LTD) that are believed to be important in mnemonic



**Figure 5.1** Schematic of a single subunit of the AMPA receptor indicating key domains. Key sites highlighted: A – alternative splice cassette generating splice variants flip and flop; B – Q/R editing site; C – binding site for interaction with proteins such as NSF and AP2; D – PDZ binding domain for interaction with PICK1 and GRIP.

processing. In addition, AMPARs further contribute to the manifestation of synaptic potentiation in that they are recruited into the post-synaptic density of those synapses that undergo potentiation.<sup>8</sup>

## Glutamate Receptors and Schizophrenia

Several mechanisms have been proposed to underlie the cognitive dysfunction associated with schizophrenia. Of these, dysfunctionality of the glutamatergic neurotransmitter system, *i.e.* “hypoglutamatergia”, has emerged as one of the strongest candidates. Extensive studies of schizophrenic patients have identified a reduction in glutamatergic neurotransmission, as measured by an imbalance in the levels of expression and functional activity of both glutamate receptors and glutamate transporters. In this respect, there is evidence for a decrease in the glutamate concentration in both CSF as well as in *post mortem* prefrontal

and hippocampal tissue from schizophrenic patients. In addition, glutamate carboxypeptidase II, an enzyme that cleaves *N*-acetyl-aspartyl glutamate (NAAG), an acidic dipeptide that acts as a glutamate receptor antagonist, is reduced in cortical areas of schizophrenia patients, leading to an increase in NAAG and a reduction in glutamate in the schizophrenic brain. mRNA for AMPARs is reduced in *post mortem* schizophrenic hippocampal tissue and both radioligand binding and immunocytochemical studies have revealed a reduction in the expression of the AMPAR itself in the medial temporal lobe of schizophrenic patients as well as alterations in the expression levels of individual subunits constituting AMPARs in prefrontal cortex, thalamus and temporal cortex, areas that have been shown to exhibit impaired activation in schizophrenic patients compared to healthy subjects when performing cognitive tasks, such as novel object recognition.<sup>9</sup>

Down-regulation of AMPARs, as occurs in schizophrenia, is likely to affect activation of the NMDA receptor. Blocking NMDA receptors impairs cognitive performance in a variety of tasks performed by rodents and non-competitive NMDA receptor antagonism, using PCP or ketamine, (a) exacerbates schizophrenic symptoms experienced by individuals already suffering from the disease and (b) induces psychotic states and disrupts cognitive performance in healthy human volunteers in a manner that more closely resembles that experienced by schizophrenic patients than is produced by an amphetamine challenge (which releases dopamine). Consistent with the role of AMPARs in cognitive function <sup>3</sup>H-AMPA binding in the dorsal hippocampus increases following learning in rats<sup>10</sup> and transgenic deletion or over-expression of individual AMPAR subunits can impair or improve learning and memory of a variety of cognitive tasks in mice.<sup>11</sup>

## 5.2 Discovery Landscape

Reinstating the loss of glutamatergic function in schizophrenia *via* alterations in AMPAR function can most easily be achieved by either direct agonism or positive modulation. Direct agonist-induced activation of AMPARs globally activates the CNS thereby losing the spatial and temporal aspects of AMPAR activation that are critical to generating a sufficient signal-to-noise ratio to enable appropriate CNS processing of sensory information. Instead positive modulation of AMPARs by, for example, slowing the rate at which the receptor (a) desensitizes in the continued presence of glutamate, or (b) deactivates after removal of glutamate, enhances and/or prolongs glutamatergic synaptic currents thereby promoting synaptic transmission and plasticity without corrupting spatial and temporal information.<sup>12</sup> As a result a number of chemically diverse AMPAR positive modulators have been developed, which potentiate AMPAR-mediated activity in *in vitro* systems, exhibiting selective potentiation at all AMPAR subunit types, compared to other ionotropic glutamate receptors, with no specificity for one particular subunit and enhancing synaptic glutamatergic activity in native tissue preparations.<sup>13</sup> Such molecules also improve cognitive performance in behavioural models in rodents,

including improvement in olfactory discrimination, radial arm maze,<sup>14</sup> conditioned fear,<sup>15</sup> water maze performance,<sup>16</sup> delayed non-match to sample,<sup>17</sup> novel object recognition and<sup>18</sup> passive avoidance.<sup>16b,18</sup> Similarly, in non-human primates improved performance has been reported in the reversal of impaired multiple schedule task,<sup>19</sup> delayed match to sample in young<sup>20</sup> and aged<sup>20a</sup> rhesus monkeys and alleviation of sleep deprivation-impaired performance of delayed match to sample.<sup>20b</sup>

Furthermore, this procognitive effect of AMPAR modulators in non-human species translates through to man. The relatively low-potency AMPAR positive modulator CX516 improved cognitive performance in small-scale double-blind clinical trials in both healthy volunteers<sup>21</sup> and elderly subjects.<sup>22</sup> In addition, this molecule also improved performance in measures of attention and memory in schizophrenic patients stabilized on the atypical antipsychotic drug clozapine,<sup>23</sup> although later trials have proved unsuccessful.

Given the wealth of evidence for a beneficial effect of AMPAR positive modulators in the treatment of cognitive dysfunction and schizophrenia, a relatively large number of clinical trials have been conducted to establish the potential of this pharmacological class in treating a range of clinical conditions. The main trials reported are delineated in Table 5.1 (with representative structures in Figure 5.2), although this list is by no means comprehensive.<sup>24</sup> The principal players are Cortex, who were co-developing Farampator (CX691, ORG24448, SCH 900460) with Organon (subsequently Schering-Plough, then Merck) for the treatment of schizophrenia and depression (phase II) although no development has been reported following the cardiac safety study in patients. Following the positive data released for CX717 in ADHD, further development has not been pursued due to the relative poor CNS exposure of this molecule. However, the further discovery of the respiratory stimulant effects of the AMPAR positive allosteric modulators on the pre-Bötzinger complex of the brain has led to continued development of an intravenous formulation of CX-717 for use alongside opioid analgesics to treat drug-induced respiratory depression. Of other note, previously Eli Lilly reported negative data for LY451395 in phase II for the treatment of AD, potentially due to selecting the wrong dose regime (0.2 mg bid over 28 days then 1 mg bid for up to 8 weeks).<sup>25</sup>

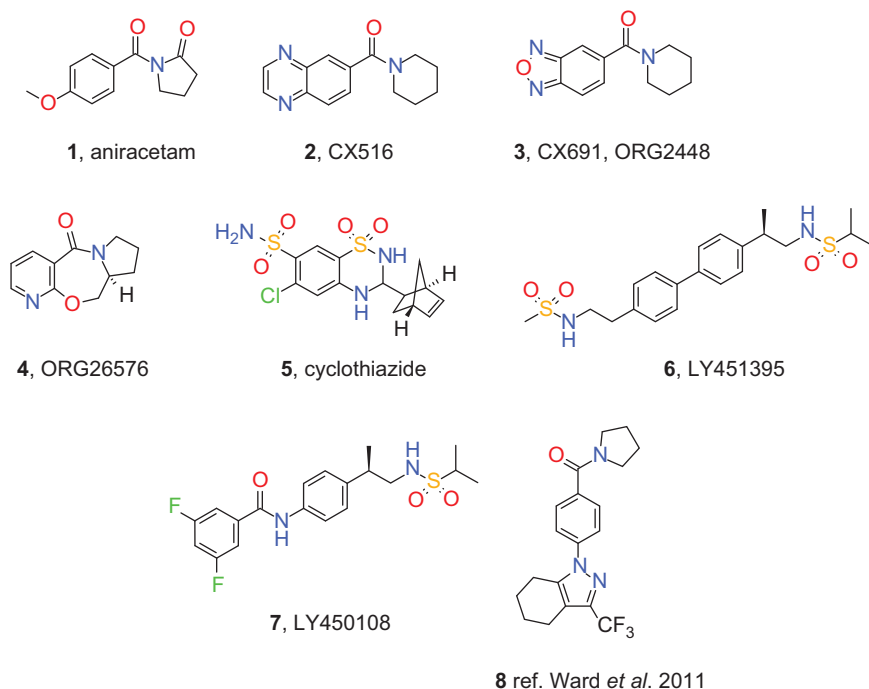
### 5.2.1 In-house Screening Methods

From awareness of the screening platforms used across the AMPAR research teams, we initially wanted to establish a versatile screening platform that would be able to support both low-volume SAR screening and also focused library to ultimately high-throughput screening. To this end, we were attracted by the approach taken by Lilly, which had delivered a distinct chemical class by high-throughput screening against recombinant human GluA4 (flip variant). Our approach was to develop similar screening methods, but we chose to establish GluA2 as the primary target as analysis of the regional brain distribution of the various GluA subunits indicated that GluA2 was expressed in more relevant brain regions for cognition and schizophrenia than GluA4.

**Table 5.1** List of clinical trials for AMPAR positive modulators.

<i>Molecule</i>	<i>Trial</i>	<i>Status</i>
<b>CX516</b> <b>Ampalex</b>	Cognitive performance in healthy young and aged volunteers (Ingvar, 1997)	Improved cognitive performance
	CX516 added to clozapine, olanzapine or risperidone in patients with schizophrenia (Goff, 2001; 2008)	Inconsistent cognitive benefits
	Efficacy and safety of CX516 in elderly participants with mild cognitive impairment	Completed
	Effects of CX516 on functioning in fragile X syndrome and autism	Completed
	Treatment of Alzheimer's disease with CX516	Completed
<b>CX691</b> <b>ORG24448</b> <b>SCH900460</b> <b>Faramaptor</b>	Cardiac safety study of PhI and PhII	95 patients
	ORG24448 patients	Completed
	Depression	180 patients
	8-week treatment, includes PET analysis	Completed
	Excluded patients with propensity for seizure following EEG assessment	
	TUNRS NIMH-funded cognitive impairments in schizophrenia	Terminated
<b>CX717</b>	8 weeks adjunctive therapy to existing atypical antipsychotic medication	
	DARPA-funded small-scale trial in healthy volunteers subjected to simulated night-shift work	No improvement in cognitive performance
	ADHD small (n = 23–28) study of 200 mg bid and 800 mg bid for 3 weeks in adults with moderate-severe symptoms	Significant improvements at highest dose on hyperactivity and inattention indices
	Drug-induced respiratory depression	
	Drug-induced respiratory depression	
	Phase II Major Depressive Disorder	Completed
<b>CX1739</b> <b>ORG26576</b>	54 patients	
	Part 1: 100–600 mg bid up to 16 d	
	Part 2: 30 subjects; high dose group and low dose group. 28 d dosing	
	ADHD	Completed
	60 patients	
	100–300 mg bid; 8 weeks	
<b>LY451395</b>	Aggression and agitation in AD	Active
	150 patients; 3 mg bid 12 weeks	
	AD	Negative
<b>GSK729327</b>	200 patients	
	PhI	Completed
	79 volunteers, 1–6 mg	

This choice created an additional complication in that, as described above, Q/R editing in TM2 of the GluA2 subunit controls the  $\text{Ca}^{2+}$  permeability of this receptor, and so the assay was configured using unedited GluA2 (Q in TM2) to allow permeability of calcium ions. Thus, human embryonic kidney (HEK)



**Figure 5.2** Representative clinical AMPAR positive modulators (CX717 not shown as structure is not yet disclosed).

cells were transfected to create an hGluA2i Q-unedited RC17 cell line. The assay was established on a fluorescent imaging plate reader (FLIPR; EMBLA instrument, Skatron), which allows a high-throughput screening platform to be easily established, by monitoring the fluorescence that is observed upon binding calcium to a Fluo-4AM (Molecular Probes) membrane permeable fluorescent dye. Furthermore, to confirm the mode of action of the compounds as allosteric potentiators rather than direct agonists or activators, a dual addition protocol was used in which a solution of the compound was assayed for 5 minutes prior to the addition of 100  $\mu$ M glutamate, which caused a dramatic increase in FLIPR counts when an AMPAR positive modulator had been pre-applied. This assay was amenable to 384-well format, and each plate contained high (150  $\mu$ M cyclothiazide + 100  $\mu$ M glutamate) and low (100  $\mu$ M glutamate) controls. This allowed the FLIPR values to be normalized to a specific percent increase from 100  $\mu$ M glutamate alone (0%) to the maximal cyclothiazide response in the presence of 100  $\mu$ M glutamate (100%).

Furthermore, to validate the output of this surrogate screen, we established a functional readout using whole-cell recordings from HEK293-hGluA2i cells using the perforated patch-clamp technique.<sup>26</sup> The cells were clamped at  $-60$  mV and control currents elicited by a fixed concentration of 3 mM glutamate were recorded prior to application of glutamate in the presence of increasing



concentrations of positive modulator (five concentrations from 10 nM to 100  $\mu$ M) in the same cell. Again, cyclothiazide was used as a positive control (at 30  $\mu$ M).

### 5.3 Case Study of Discovery Project – Background

Our target candidate profile for this discovery project was a molecule that was a potent potentiator of AMPA-mediated responses *in vitro* and *in vivo* and that was able to demonstrate downstream consequences of this activation through enhancing cognitive abilities in preclinical models of learning and memory. Furthermore, the candidate needed to possess all the standard attributes of a CNS-acting drug, which would be dosed at least once daily for a prolonged period of time, would be compatible with standard medications, in particular all existing antipsychotic medications, and would be safe and well tolerated. We were aware at the initiation of this project that the nature of the AMPA receptor and our desired mode of action would render standard translational approaches very challenging. In particular, we wanted to explore biomarker, imaging and surrogate readouts that would facilitate the transition from the research project to clinical development.

Ahead of the high-throughput screening campaign that led to the discovery of the development candidate **8**, 1-[4-(1-pyrrolidinylcarbonyl)phenyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazole,<sup>27</sup> we ran a number of exploratory efforts to seek to improve the overall drug-like properties and/or tolerability of molecules that had been publically disclosed. To this end, we investigated the phenethylsulfonamide class with a view to improving physicochemical parameters, in particular to maximize unbound brain concentrations and/or improve the margin between efficacy and tolerability (for studies in related chemical series see ref. 28).

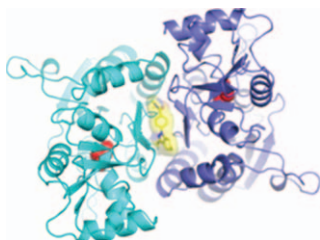
### 5.4 Case Study of Discovery Project – Crystallography

During the early discovery phase of this project we were attracted by the potential of the work published from Eric Gouaux's laboratory involving crystallographic and mechanistic studies on the ligand-binding domain of the GluA2 subunit.<sup>8,29</sup>

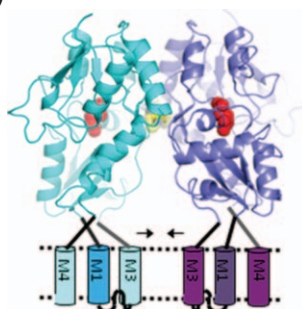
These structures were obtained by producing constructs of the ligand-binding domain that were amenable to crystallization, by ligating the amino acid sequences connected to the M1 and M3 transmembrane domains with a short peptide bridge. These studies enabled us to take the decision to produce in-house crystal structures of the ligand-binding domain construct of GluA2 and we prepared the pET15b-ratGluA2flopS1-GlyThr-S2 N754S construct as previously described.<sup>27</sup> This expressed construct successfully generated the desired protein, which, following a screen of crystallization conditions, afforded appropriate material from which to collect high-resolution structural X-ray data. These data were processed using CCP4 suite software<sup>30</sup> to give structures that were refined with Refmac<sup>31</sup> using the programme Coot<sup>32</sup> for the actual model building.

Figure 5.3 contains pictures representing well-characterized AMPAR modulators as well as an intermediate fragment **6a** from the phenylethylsulfonamide

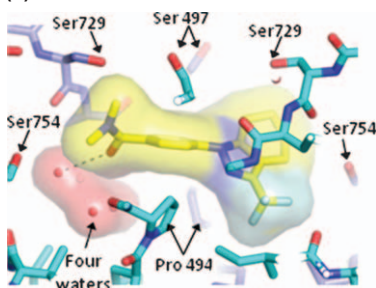
(a)



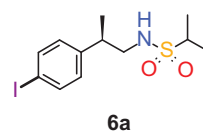
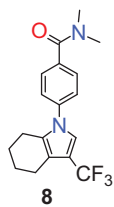
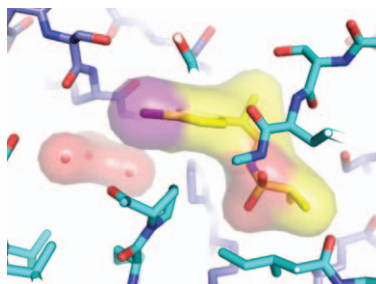
(b)



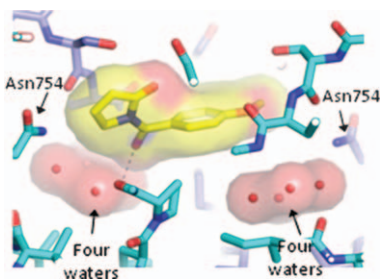
(c)



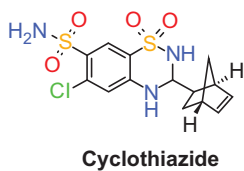
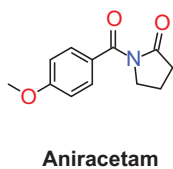
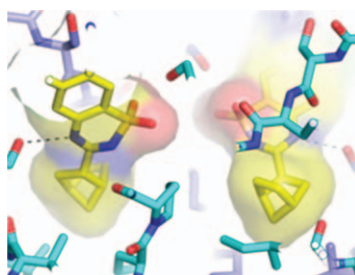
(d)



(e)



(f)





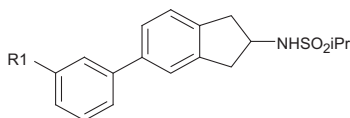
series. As can be seen from panel a, the modulators bind at the dimer interface with each dimer subunit binding a glutamate molecule (modulator shown in yellow; glutamate in red). Panel b gives a clearer orientation of the subunits relative to the components of the sequence that would have linked to the transmembrane regions. The key potential hydrogen bonding residues are highlighted in panel c for molecule **8** (pdb code: 2xx8), which we identified later in the lifetime of this project. It can be seen that this molecule reveals a similar binding mode to many published AMPAR positive modulators<sup>33</sup> in that it binds to the 2-fold axis of the dimer. The structure shown in panel d contains an iodophenyl fragment **6a**, which was used for Suzuki coupling exploration of biaryl molecules such as **6** mentioned earlier.<sup>34</sup> Interestingly, this molecule retained some AMPAR potency, and this structure was used for generation of new analogue ideas described below. For comparison with other known molecules, the original nootropic agent aniracetam (pdb code: 2al5) and the standard AMPAR positive modulator cyclothiazide (pdb code: 1lbc) are included in panels e and f, respectively. For both of these structures, we see that the binding pocket resembles an inverted U, which, as for **8**, generally binds the positive modulator across the dimer interface. Clearly, the symmetry of this system creates two identical binding pockets by dimerization of the protein units, and this created an additional complication when solving the crystal structure data, in that the difference density of the ligand is recorded for our ligands in two orientations requiring additional data processing to deconvolute. Interestingly, however, in the aniracetam structure both ends of the pocket are occupied by a cluster of four water molecules (small red spheres) and this remains the only molecule to bind to the protein and not occupy the classical binding pocket. In contrast, cyclothiazide binds with different stoichiometry with two molecules present in the crystal structure, in the classic binding pocket, but not occupying the intervening territory occupied by aniracetam.


## 5.5 Case Study of Discovery Project – Hit to Lead Optimization

As discussed, our initial explorations focused on the indane sulfonamide template and, in particular, around optimization of the 3-phenyl substituent

**Figure 5.3** X-ray crystal structures of AMPAR positive modulators bound to ligand binding domain construct of GluA2 S1S2 LBD. (a) Structure of GluA2 S1S2 ligand binding domain construct showing modulator **8** (yellow) bound at the dimer interface in the presence of 2 glutamate molecules (red). (b) Schematic of the ion channel in the open state (arrows indicating direction of movement for closure). (c–f) Comparison of binding modes of structurally distinct AMPAR positive modulators in complex with GluA2S1S2 LBD all binding at dimer interface with molecule carbon backbone shown in yellow, water molecules as red spheres and H-bonds as dotted red lines. Structure c=**8**, d=**6a**, e=aniracetam and f=cyclothiazide. Residue labelling is consistent between structures other than for residue 754 which is Asn in the flop splice variant and Ser in the flip splice variant.

**Table 5.2** Biological activity and physiochemical data for 3-substituted phenyl indane analogues. FLIPR generated  $pEC_{50}$  against hGluA2 flip isoform. (Asym max is the fitted maximum response, relative to 100% defined as the maximal response of cyclothiazide standard.); clogP Daylight Chemical Information Systems Inc., Aliso Viejo, CA, <http://www.daylight.com>; Rat plasma protein binding (rat PPB) values were determined using a 96-well plate equilibrium dialysis method at a concentration of 1  $\mu$ g/ml.



Compound	R1	$pEC_{50}$	Asym max	PSA ( $\text{\AA}^2$ )	clogP	%rat PPB
<b>9</b>	NHSO <sub>2</sub> Me	6.1	123%	92	2.7	95.9
<b>10</b>	SO <sub>2</sub> NMe <sub>2</sub>	5.1	30%	84	3.0	—
<b>11</b>	COMe	<4.3	31%	63	3.3	—
<b>12</b>	CH <sub>2</sub> COMe	5.2	75%	63	3.1	—
<b>13</b>	NHCOMe	4.7	54%	75	3.6	94.9
<b>14</b>	NHSO <sub>2</sub> Et	5.6	101%	92	3.2	—
<b>15</b>	NMeSO <sub>2</sub> Me	5.9	105%	84	2.7	96.4
<b>16</b>		6.4	118%	84	2.8	96.7

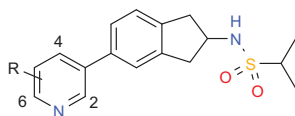
to balance required properties. As shown in Table 5.2, our lead molecule in this area, *meta*-phenylsulfonamide **9**, was a potent potentiator of the AMPAR, reaching a greater degree of potentiation than the cyclothiazide standard response. SAR within this series was consistent with that described for other phenyl sulfonamides in that the secondary isopropyl sulfonamide unit was essential for high potency and molecules altering the alkyl chain length were less effective, whereas replacements to the sulfonamide or substituents on the nitrogen led to a loss of activity. Although **9** represented a potent lead, it is clear that the overall polar surface area is likely to be in the range observed for poorly CNS-penetrant molecules. This was confirmed by a rat pharmacokinetic study in which **9** was dosed orally at 3 mg/kg, affording excellent systemic exposure but a brain:blood ratio of only 0.1. Further data confirmed that the molecule was highly permeable but significantly effluxed in MDCKII-MDR1 cells heterogeneously expressing human P-gp (ER 5.8 data generated relative to the potent P-gp inhibitor GF120918). Clearly, the secondary sulfonamide group is a highly polar motif and as modification to the indane sulfonamide was not possible, we explored alternatives to the *meta*-phenyl sulfonamide substituent. In contrast to the indane sulfonamide group, it was possible both to identify alternative groups replacing the sulfonamide that retained activity and also to substitute on the secondary nitrogen without impacting potency.

Substitution alone, to afford molecules such as **14–16**, led to potent potentiators, with reduced, but still elevated PSA values. For proof of this, molecule **15** was investigated in the same rat pharmacokinetic and P-gp efflux models and found to be improved (brain:blood 0.4; efflux ratio 3.2 compared to 5.8 for **9**). These findings were a clear indication that the polar surface area required further reduction, although, unfortunately, replacement of the sulfonamide with inherently less polar groups led, for those groups that generated a PSA < 70, to significantly less potent molecules. The few molecules that did give reasonably good pEC<sub>50</sub> values, such as **12**, were inherently metabolically unstable.

Given the lack of success in balancing AMPAR activity with PSA for the molecules in Table 5.2, which actually represents just a subset of exemplars prepared and characterized, we decided to investigate replacement of the phenyl ring with heterocyclic rings – in part to fish for additional hydrogen bonding interactions, but principally to look to reduce the lipophilicity and molecular weight of the molecules and, by so doing, to be able to increase the unbound drug concentrations that would be achievable.

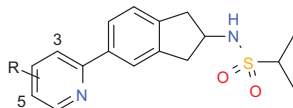
The most promising heterocycle by far was pyridine, which gave active molecules for all regioisomeric forms and also for a range of substituents. Most promisingly, 2- and 3-pyridyl indanes afforded a range of active molecules with low PSA values although, as is immediately apparent from Tables 5.3 and 5.4, this change of indane substituent from the sulfonamido-phenyl group has been

**Table 5.3** Biological and *in vitro* DMPK profiling of novel 3-pyridyl indane analogues. Molecules shown as *rac* (racemic) or as defined single stereoisomers. FLIPR generated pEC<sub>50</sub> against hGluA2 flip isoform. (Asym max is the fitted maximum response, relative to 100% defined as the maximal response of cyclothiazide standard.); clogP Daylight Chemical Information Systems Inc., Aliso Viejo, CA, <http://www.daylight.com>. For values represented by \*, no value could be measured.



Compound	R		pEC <sub>50</sub>	Asym max %	PSA (Å <sup>2</sup> )	MWt	clogP	pK <sub>a</sub>
<b>17</b>	H	rac	4.8	101	59	316	2.3	5.1
<b>18</b>	2-F	rac	4.8	96	59	334	2.6	*
<b>19</b>	6-F	rac	5.3	106	59	334	2.6	*
<b>20</b>	2,6-diMe	rac	4.5	59	59	344	3.0	6.7
<b>21</b>	6-F	<i>R</i>	< 4.5	*	59	334	2.6	*
<b>22</b>	6-F	<i>S</i>	5.6	107	59	334	2.6	*
<b>23</b>	6-Me	<i>S</i>	5.7	104	59	330	2.8	5.8

**Table 5.4** Biological and *in vitro* DMPK profiling of novel 2-pyridyl indane analogues. Molecules shown as *rac* (racemic) or as defined single stereoisomers. FLIPR generated  $pEC_{50}$  against hGluA2 flip isoform. (Asym max is the fitted maximum response, relative to 100% defined as the maximal response of cyclothiazide standard.); clogP Daylight Chemical Information Systems Inc., Aliso Viejo, CA, <http://www.daylight.com>.



Compound	R		$pEC_{50}$	Asym max %	PSA ( $\text{\AA}^2$ )	MWt	clogP	$pK_a$
<b>24</b>	5-F	rac	5.2	114	59	334	2.8	2.7
<b>25</b>	5-Me	rac	5.5	90	59	330	3.0	4.9
<b>26</b>	5-F	<i>S</i>	5.7	103	59	334	2.8	2.7

achieved at the cost of AMPAR potency. However, although the unsubstituted 3-pyridyl unit was only weakly active, substitution with methyl or halogen groups was able, for specific substitution patterns, to increase target potency. We also took advantage of this series to investigate the stereochemistry of the indane ring chiral centre and prepared a number of these analogues in both *R* and *S* forms. This was generally accomplished by starting from the product of a direct bromination of 2-aminoindane (a reaction that we later achieved on considerably larger scale to support phase I clinical and supporting studies), *via* a classic resolution with either enantiomeric form of camphor sulfonic acid. The *R* or *S* 6-bromo-2-aminoindane was then elaborated using standard sulfonamide formation followed by Suzuki coupling chemistry to obtain the single enantiomer final products exemplified by **21–23** and **26**. As an aside, although this chemistry appeared straightforward, both of these steps required considerable effort to become fully optimized. Initially, the sulfonamide formation was inefficient across a range of standard conditions investigated, and our optimization eventually selected the use of DBU (1,8-diazabicyclo [5.4.0]undec-7-ene) as base, which was then able to achieve near quantitative conversion. The subsequent Suzuki coupling reaction, whilst giving us great versatility for the synthesis of focused SAR array sets and which could be achieved with either possible coupling partner combination (*i.e.* the boronic ester present on either the heterocycle or the indane), nonetheless afforded low yields of the target molecules for specific substituted pyridine coupling partners. Optimization of these reaction conditions proved particularly challenging, especially on scale-up.

However, from these efforts, our work to prepare the single enantiomer molecules clearly indicated that the *S* enantiomer was essentially the only active species, and allowed **22**, **23** and **26** almost to achieve the target sub-micromolar potency.

A component of the willingness to explore this lower target potency lay in the observations that we were generating in parallel with this lead optimization, by running studies to validate our screening cascade through characterization of a range of compounds in downstream models. As detailed above, in addition to the FLIPR-based primary screen measuring calcium influx through the channel in a recombinant cell line, we also were screening using whole-cell patch clamp electrophysiology in the same cell line for a more direct functional readout. Understandably, both of these techniques bear inherent deficiencies when considering their direct relevance to the intact receptor *in vivo*, and so we also implemented the routine use of whole-cell patch clamp electrophysiology using rat hippocampal neuronal cells. This native tissue preparation should contain AMPA receptors comprising a heterogeneous mixture of subunits and splice variants, which, furthermore, should be present with their associated accessory proteins, which are being increasingly recognized as major regulators of AMPAR activity.<sup>35</sup> With these differences, it came as no surprise that the activity observed in the GluA2 recombinant cell line electrophysiology was not directly reproduced by the native tissue assay. In particular we observed varying profiles of deactivation and desensitization, and generally observed a ten-fold increased sensitivity in the rat neuronal culture preparation.

Furthermore, our growing in-house data sets made it increasingly clear that there was not a simple correlation between the readouts of our various assay systems. Specifically we identified a lack of linearity between the data from primary screening (FLIPR on recombinant homomeric GluA2 flip receptors), secondary screening (electrophysiology on either recombinant or native cells) and *in vivo* screening in pharmacodynamic or behavioural models. Consequently, we decided to sacrifice a measure of activity in our primary assay to achieve the potential to capture greater absorption, CNS penetration and free fraction levels.

As can be seen from Table 5.4, the pyridines gave very favourable CNS drug-like properties in terms of molecular weight, clogP and PSA, as well as metabolic stability, *in vitro* DMPK, P450, selectivity, permeability and, particularly for those for which we could measure a  $pK_a$ , solubility. These properties were confirmed in some preliminary rat pharmacokinetic studies, for which the exemplars profiled distributed preferentially into the brain and did not interact with P-gp. These data are highlighted in Table 5.5 for what became our three late lead optimization pre-candidate molecules.

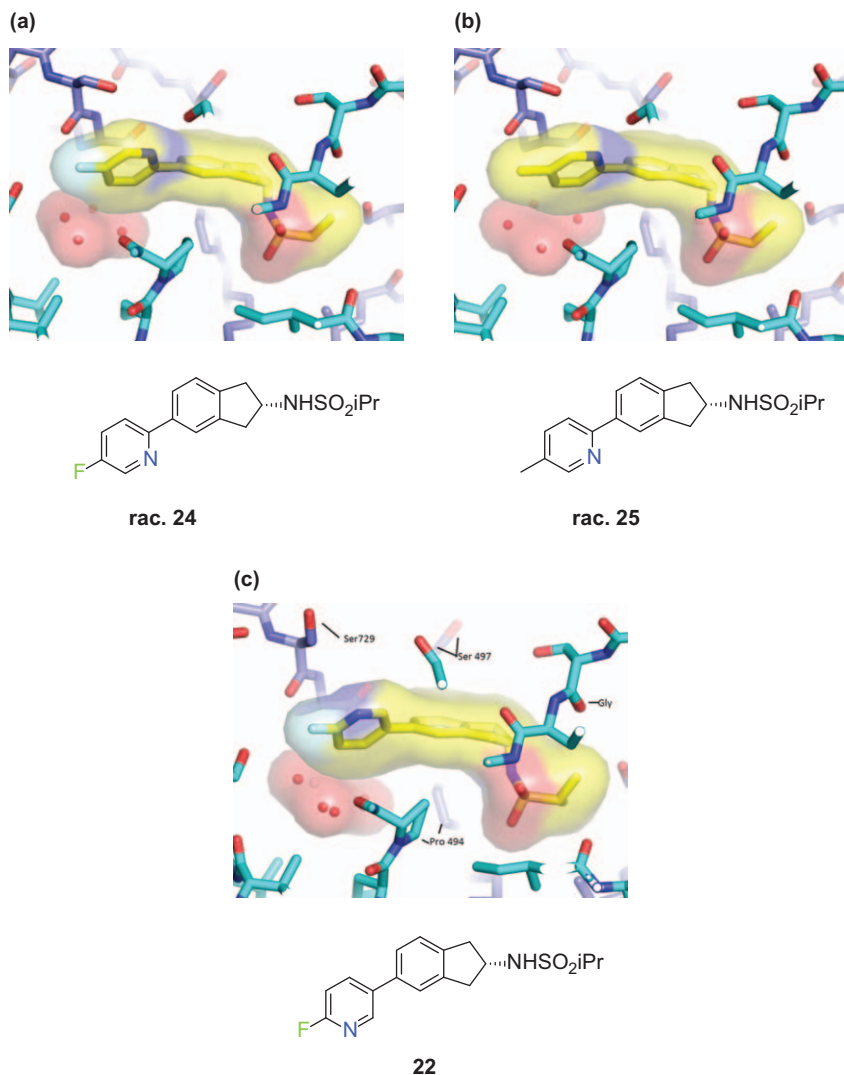
**Table 5.5** Preclinical species DMPK profiling of lead pyridyl indane analogues in rat (1 mg/kg iv; 3 mg/kg po serial and composite profiles).

	Blood clearance (ml/min/kg)	$T_{1/2}$ (h)	$V_{ss}$ (l/kg)	Oral $C_{max}$ (ng/ml)	$F$ (%)	Brain:blood $AUC_{0-t}$ ratio
<b>22</b>	5.4	5	2.4	416	61	2.1
<b>23</b>	55	0.6	2.4	116	25	1.3
<b>26</b>	24	1	2.2	436	72	1.4

Due to the challenges of translating from the *in vitro* biological characterization to activity in the behavioural models, we decided to progress a number of early promising leads in parallel through the downstream cascade – in particular to demonstrate efficacy at concentrations with a significant window of tolerability. Specifically, we were concerned about the potential for excitotoxicity or convulsion with any mechanism that potentiates excitatory neurochemical signalling. Our in-house profiling of several of the AMPA compounds that had entered clinical trials had revealed a propensity for overt convulsant or pro-convulsant liabilities. Clearly, there are many reasons that individual research groups and in particular pharmaceutical companies would be reluctant to associate these liabilities with the mechanism of AMPAR potentiation, but nonetheless, despite the vast literature surrounding this mechanism of action and these aforementioned molecules, the topic of this potential liability is broadly avoided. However, for us to progress any molecule into clinical evaluation, we clearly needed to first and foremost build confidence in the safety of our potential medicines. To that end, we used the maximal electroshock threshold test (MEST test) to compare the profile of our AMPAR positive modulators to the known pro-convulsant standard, picrotoxin (used as a positive control 2.0 mg/kg was administered ip 30 min before testing). Testing consisted of assessing the induction of a tonic hind limb extensor seizure following a 0.1 second shock administered *via* corneal electrodes according to the “up and down” method.<sup>36</sup> In all experiments a separate group of three animals per dose group were dosed and blood and brain samples taken at the appropriate pre-treatment time for analysis of compound levels. These data were key for us to understand the properties of our AMPAR positive modulators relative to their pharmacodynamic and behavioural readouts, and whilst the propensity of molecules to elicit convulsant or pro-convulsant activity could be clearly linked to the product of their *in vitro* potency and achieved free brain concentrations, the correlation was not simple and required detailed characterization of the mechanism of potentiation using various electrophysiological techniques. More revealingly, it appeared that those molecules that had particularly high AMPAR potency in the primary FLIPR assay (which were generally those molecules able to make additional interactions with the AMPA modulator binding site) elicited pronounced effects in the MEST test at elevated doses, without necessarily demonstrating the compensatory enhanced efficacy at lower concentrations in the cognition assays.

## 5.6 Crystallographic Studies

Following the earlier work to validate the in-house crystallography we developed the routine use of X-ray crystallography within our lead optimization project. However, as the primary constraints within this chemical series were the physicochemical and pharmacokinetic parameters, we did not require the iterative information from structural studies to direct the new targets as critically as we did in the phase of the project that identified development



**Figure 5.4** X-ray crystal structures of AMPAR positive modulators bound to ligand-binding domain construct of GluA2 S1S2 LBD. Structures show modulators all bound to protein dimer interface with carbon backbone in yellow and water molecules as red spheres. Structures shown for (a) *S* enantiomer of racemic **24**, (b) *S* enantiomer of racemic **25** and (c) single enantiomer **22**.

candidate 8.<sup>27</sup> Nonetheless, a number of molecules were characterized and their representative structures are shown in Figure 5.4.

Taking our lead structure **22**, the 1.55 Å structure (pdb code: 2xhd) reveals a similar binding mode to the structures reported earlier. Consistent with the



iodophenyl fragment above as well as later reports<sup>37</sup> for other molecules bearing a phenethylsulfonamide group, the isopropyl sulfonamide unit is buried deep into a pocket at the dimer interface. This mode of binding was conserved across the range of indane substituents investigated, as demonstrated by the pictures of the racemic forms of compounds **24** and **25**.

## 5.7 Characterization of Preclinical Development Candidate

As mentioned above, a range of molecules was profiled through downstream assays to select finally the single molecule to progress into clinical development. Rather than detail the full account of this comparative profiling, a summary of the various aspects investigated will suffice to demonstrate those data necessary to progress an AMPA receptor positive modulator into phase I.<sup>38</sup>

The molecule that finally met all our required criteria, **22**, demonstrated a  $pEC_{50}$  and maximum potentiation values (relative to the established AMPAR positive modulator cyclothiazide ( $150\ \mu\text{M}$ )) of  $5.57 \pm 0.07$  and  $100.7 \pm 3.9$ , respectively, in the functional activity at recombinant human GluA2i homomeric AMPARs using FLIPR methodology. When applied alone, in the absence of glutamate, **22** did not affect intra-cellular  $\text{Ca}^{2+}$  levels indicating that it possessed no intrinsic agonist activity. This potentiation was in line with electrophysiological evaluation, which was performed using the HEK293-hGluA2i stable cell line (as above). This approach employed a fast perfusion system to produce a more rapid application and removal of glutamate than is achievable in the FLIPR assay, thereby more closely mimicking physiological activation of AMPARs. Using this approach, **22** potentiated glutamate-induced whole-cell, AMPAR-mediated currents producing a maximal response that amounted to  $112 \pm 32\%$  of the maximal response induced by the reference AMPAR positive modulator cyclothiazide ( $30\ \mu\text{M}$ ) with a  $pEC_{50}$  value of  $5.19 \pm 0.02$  in close agreement to that generated using the FLIPR/ $\text{Ca}^{2+}$  influx assay.

We also wanted to demonstrate the activity of our AMPAR positive modulators across the range of AMPA subtypes and so we generated transfected cell lines for GluA1, GluA3 and GluA4 flip variants, as well as GluA2 flop splice variant, and used these to generated data in the above FLIPR assay across a range of structures. For the molecules we characterized, there was a less than 10-fold difference between  $pEC_{50}$  values for all hGluAi homomeric AMPARs, indicating that our molecules possess little specificity for individual GluA subunits. The difference between the  $pEC_{50}$  value for rat GluA2i AMPARs and the different hGluA AMPARs was also less than 10-fold.

One of the recurrent issues we faced in progressing molecules from this project was to determine appropriate means to assess selectivity, given the relatively low  $pEC_{50}$  values for potentiation of the receptor. Selectivity within the receptor class was demonstrated by showing a lack of activity as opener or blocker of NMDA receptors (NMDAR1/2B) as well as an absence of agonist,



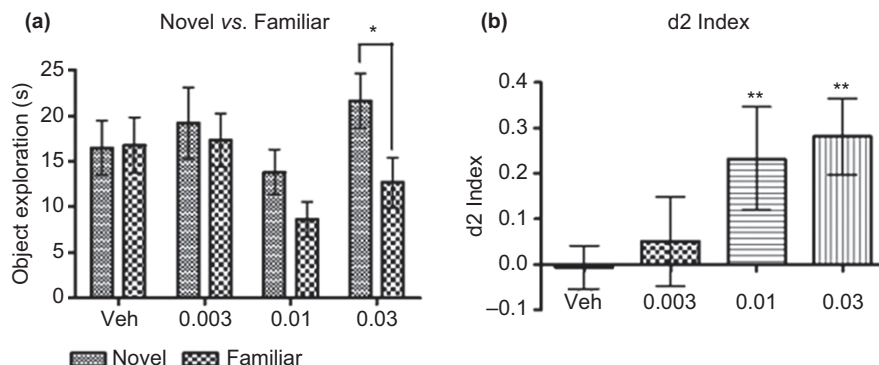
positive modulator or antagonist activity at kainate receptors (GluK1, Q-edited). These data were confirmed by evaluation of the effects of molecules including **22** using NMDA- and kainate-induced whole-cell currents in rat cultured hippocampal neurons. Secondly, in-house cross screening and subsequently wider CEREP receptor profiling against a full panel of available proteins was used for broader identification of potential liabilities – and importantly, the data generated were compared to the targeted clinical concentrations derived from the downstream cognition models, rather than the absolute pEC<sub>50</sub> against the AMPAR.

AMPA positive modulators are reported to be effective in a range of models of learning and memory. We were able to demonstrate similar activity with molecules identified from the literature, and also subsequently with our own molecules. For our lead molecule **22**, we demonstrated enhanced cognitive effects in a number of rat models including novel object recognition (NOR), Morris water maze in aged rats and scopolamine-induced deficits in passive avoidance. We made greatest use of the data generated in the novel object recognition assay, in which impairment was created by time delay rather than using a pharmacological agent such as scopolamine, and relied on this model to discriminate between the effective concentrations of our molecules.

In our NOR assay, the rats were exposed to an arena (for three minutes) containing two novel objects. After 24 hours the rats were returned to the arena containing one of the original objects and an additional novel object and the time the rat spent exploring the two objects was recorded. As rats have an inherent propensity to explore novel objects over familiar ones, the time recorded exploring the novel and the familiar object can be used to infer how well the rats recall the familiar object from the first exposure to the test arena.

In our studies many of our molecules showed robust activity within the NOR model. For our lead **22**, we saw that dosing four hours prior to both test arena presentations gave a bell-shaped dose response curve in which an improvement in object recognition was observed at 0.3 and 1.0 mg/kg but lost effect either at lower doses (0.1 mg/kg) or at higher doses (10 mg/kg). This bell-shaped phenomenon was frequently observed for this behavioural model across various pharmacological mechanisms.

From literature reports on improved efficacy with AMPA positive modulators following sub-chronic dosing, we decided to investigate this within the NOR model, which would additionally discharge the risk of adaptive tolerance to repeated receptor potentiation. For this paradigm, **22** was dosed for seven days once daily in advance of the test protocol described above. This sub-chronic dosing regimen led to a marked shift in the dose response, leading to robust and reproducible efficacy at 0.03 mg/kg, with significant effects on the d2 index (proportion of time exploring the novel object in the second exposure to the test arena) at 0.01 mg/kg (Figure 5.5). Satellite animals were used to determine compound concentrations, which were linearly proportional to the administered dose, and gave, for the sub-chronic model, an effective concentration of 7 ng/ml in blood, 11 ng/g in brain.



**Figure 5.5** a) Effect of **22** in the novel object recognition assay after 7 days sub-chronic administration, once daily dosing of **22** followed by dosing 4 h prior to T1 (exposure to two identical objects) then after 24 h, dosing 4 h prior to T2 (exposure to one novel and one familiar object). b) Graph showing y-axis representing d2 index (proportion of time exploring the novel object) vs. x-axis representing dose administered orally (mg/kg) in rat. For both, significance is labelled \* $P < 0.05$  and \*\* $P < 0.01$  compared to vehicle treated rats.

In addition to these encouraging data, we also wanted to establish a stronger link between our *in vitro* assays and our behavioural assays, in particular to gain greater confidence that our observations of enhanced cognition were driven by the AMPAR, for which a direct measure of target engagement was not straightforward. To this end, we evaluated **22** in an *in vivo* model of electrophysiology assessing the potentiation of electrically evoked AMPAR-mediated synaptic potentials recorded from the dentate gyrus of the anaesthetized rat. Using this model, and following stimulation of the medial perforant pathway we observed a population spike in the hippocampal dentate gyrus granule cell layer after dosing **22** at 0.1 mg/kg intravenously (population spike amplitude of 18%). The brain samples taken immediately after measurement of the population spike showed **22** present at 198 ng/g, which, correcting for protein binding, gave an estimated free concentration of 5.1 ng/g or 15 nM, which is in close agreement with the *in vitro* electrophysiology data reported earlier.

For further confidence in the mode of action of our molecules, we performed microdialysis studies, and observed increased extra-cellular levels of acetyl choline in the cingulate cortex and dorsal hippocampus as well as dopamine. There was no change in either serotonin or noradrenaline concentration.

As part of the liability profile mentioned previously, **22** was profiled in the maximal electroshock threshold test with acute and sub-chronic dosing. On acute dosing, **22** significantly decreased the seizure threshold at 30 mg/kg and 100 mg/kg, the former leading to a brain concentration of 3,390 ng/g in the brain.

## 5.8 Clinical Characterization of Development Molecule

Following the preclinical data package described as well as associated data, which increased our confidence in the developability of **22**, we progressed it through the standard package of preclinical toxicology studies. In particular, we demonstrated that **22** had no evidence of genotoxic potential and no significant cardiovascular risks from a study in cynomolgus monkey and from a hERG electrophysiology assay. Screening for six weeks' duration allowed us to establish no adverse event levels with an acceptable safety margin for the doses we wished to explore clinically. The identification of clinical doses is challenging, requiring the product of scaling the pharmacokinetic properties from preclinical to clinical species, with a clear PK-PD relationship in an appropriate model (allowing for corrections for species' orthologue and protein binding differences). For the former, we had observed a consistently good pharmacokinetic profile across species and, coupled with the high permeability, lack of transporter protein interactions and good CNS penetration in preclinical species, we expected to see a similarly good pharmacokinetic profile in man. Generation of a robust pharmacokinetic-pharmacodynamic relationship is more challenging, and we relied on the effective concentrations measured in the various NOR tests across different dosing frequencies and time point assessments.

For progression of **22** into a first time in human studies in healthy volunteers, we explored single doses in the 0.25–6.0 mg dose range over 64 subjects. We also dosed a multiple dose of 0.1 mg in 15 subjects for 28 days (once daily administration). These evaluations concluded that **22** was well tolerated and neither safety issues nor adverse events or withdrawals were observed. From determination of the pharmacokinetics, **22** was rapidly adsorbed ( $C_{\max}$  0.5–3 h) with a long apparent half-life (average 107–168 h), which was surprising in light of the preclinical findings. Dose escalation proved that the pharmacokinetic profile increased linearly.

## 5.9 Conclusions and Future Perspectives

The development of new medicines exploiting potentiation of the AMPAR has been frustratingly slow, which is in part due to the complexities of linking the underlying mode of action at the receptor to the pharmacodynamic response and in part due to the challenge of running informed trials in which the clinical outcome is clearly associated with a given level of target engagement. Recently, an instructive fuller account of the derivation of a pharmacokinetic-pharmacodynamic relationship has been described for ORG 26576 and was used to predict human target engagement and clinical dosing.<sup>39</sup> However, more translational techniques have not yet proved effective in guiding patient-based clinical studies. The case study outlined in this chapter highlights some of the challenges of understanding the fundamental mechanisms involved in receptor potentiation, and how the pragmatic progression of a discovery project requires incremental confidence building to iterate to a clinical development candidate.

Hopefully, the increased number of available clinical AMPAR positive modulator tools will drive the successful progression of a potentiator into large-scale phase III trials.

## Acknowledgements

I would like to thank Dr Mark Harries for contributions to the text and Dr Benjamin Bax (GlaxoSmithKline, Stevenage, UK) for generating the protein-ligand complex graphics.

## References

1. a) C. Zarate, R. Machado-Vieira, I. Henter, L. Ibrahim, N. Diazgranados and G. Salvadore, *Harvard Review of Psychiatry*, 2010, **18**, 293–303; b) G. Sanacora, C. A. Zarate, J. H. Krystal and H. K. Manji, *Nat. Rev. Drug Discov.*, 2008, **7**, 426–437.
2. M. Laruelle, W. G. Frankle, R. Narendran, L. S. Kegeles and A. Abi-Dargham, *Clin. Ther.*, 2005, **27**(A), S16–24.
3. H. Uchida, H. Takeuchi, A. Graff-Guerrero, T. Suzuki, K. Watanabe and D. C. Mamo, *J. Clin. Psychopharmacol.*, 2011, **31**, 497–502.
4. J. J. Hagan and D. N. Jones, *Schizophr. Bull.*, 2005, **31**, 830–853.
5. a) J. W. Young, S. B. Powell, V. Risbrough, H. M. Marston and M. A. Geyer, *Pharmacol. Ther.*, 2009, **122**, 150–202; b) M. Baharnoori, C. Bartholomeusz, A. A. Boucher, L. Buchy, C. Chaddock, B. Chiliza, M. Föcking, A. Fornito, J. A. Gallego, H. Hori, G. Huf, G. A. Jabbar, S. H. Kang, Y. El Kissi, J. Merchán-Naranjo, G. Modinos, N. A. M. Abdel-Fadeel, A.-K. Neubeck, H. P. Ng, G. Novak, O. O. Owolabi, D. P. Prata, N. P. Rao, I. Rieckens, D. C. Smith, R. P. Souza, R. Thienel, H. D. Trotman, H. Uchida, K. A. Woodberry, A. O'shea and L. E. DeLisi, *Schizophr. Res.* 2010, **124**, e1–e62.
6. G. L. Collingridge, R. W. Olsen, J. Peters and M. Spedding, *Neuropharmacology*, 2009, **56**, 2–5.
7. P. E. Chen and D. J. Wyllie, *Br. J. Pharmacol.*, 2006, **147**, 839–853.
8. a) Y. Sun, R. Olson, M. Horning, N. Armstrong, M. Mayer and E. Gouaux, *Nature*, 2002, **417**, 245–253; b) E. Gouaux, *J. Physiol.*, 2004, **554**, 249–253.
9. a) S. Akhondzadeh, *J. Clin. Pharm. Ther.*, 1998, **23**, 243–246; b) K. Hashimoto, N. Okamura, E. Shimizu and M. Iyo, *Curr. Med. Chem.: Cent. Nerv. Syst. Agents*, 2004, **4**, 147–154.
10. M. Cammarota, L. R. Bevilacqua, J. S. Bonini, J. I. Rossatto, J. H. Medina and N. Izquierdo, *Neurotox. Res.*, 2004, **6**, 205–212.
11. a) D. M. Bannerman, R. M. Deacon, P. H. Seeburg and J. N. Rawlins, *Behav. Neurosci.*, 2003, **117**, 866–870; b) A. N. Mead, G. Brown, J. Le Merrer and D. N. Stephens, *Psychopharmacology (Berl.)*, 2005, **179**, 164–171; c) W. B. Schmitt, R. Sprengel, V. Mack, R. W. Draft, P. H. Seeburg,

- R. M. Deacon, J. N. Rawlins and D. M. Bannerman, *Nat. Neurosci.*, 2005, **8**, 270–272; d) D. R. Shimshek, T. Bus, J. Kim, A. Mihaljevic, V. Mack, P. H. Seeburg, R. Sprengel and A. T. Schaefer, *PLoS Biol.*, 2005, **3**, e354.
12. S. E. Ward, B. D. Bax and M. Harries, *Br. J. Pharmacol.*, 2010, **160**, 181–190.
13. a) P. Miu, K. R. Jarvie, V. Radhakrishnan, M. R. Gates, A. Ogden, P. L. Ornstein, H. Zarrinmayeh, K. Ho, D. Peters, J. Grabell, A. Gupta, D. M. Zimmerman and D. Bleakman, *Neuropharmacology*, 2001, **40**, 976–983; b) Y. F. Xia, M. Kessler and A. C. Arai, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 277–285.
14. U. Staubli, G. Rogers and G. Lynch, *Proc. Natl Acad. Sci. USA*, 1994, **91**, 777–781.
15. M. T. Rogan, U. V. Staubli and J. E. LeDoux, *J. Neurosci.*, 1997, **17**, 5928–5935.
16. a) I. Zivkovic, D. M. Thompson, M. Bertolino, D. Uzunov, M. DiBella, E. Costa and A. Guidotti, *J. Pharmacol. Exp. Ther.*, 1995, **272**, 300–309; b) J. C. Quirk and E. S. Nisenbaum, *CNS Drug Rev.*, 2002, **8**, 255–282.
17. R. E. Hampson, G. Rogers, G. Lynch and S. A. Deadwyler, *J. Neurosci.*, 1998, **18**, 2748–2763.
18. C. Lebrun, E. Pilliere and P. Lestage, *Eur. J. Pharmacol.*, 2000, **401**, 205–212.
19. D. M. Thompson, A. Guidotti, M. DiBella and E. Costa, *Proc. Natl Acad. Sci. USA*, 1995, **92**, 7667–7671.
20. a) J. J. Buccafusco, T. Weiser, K. Winter, K. Klinder and A. V. Terry, *Neuropharmacology*, 2004, **46**, 10–22; b) L. J. Porrino, J. B. Daunais, G. A. Rogers, R. E. Hampson and S. A. Deadwyler, *PLoS Biol.*, 2005, **3**, e299–e299.
21. M. Ingvar, J. Ambros-Ingerson, M. Davis, R. Granger, M. Kessler, G. A. Rogers, R. S. Schehr and G. Lynch, *Exp. Neurol.*, 1997, **146**, 553–559.
22. G. Lynch, R. Granger, J. Ambros-Ingerson, C. M. Davis, M. Kessler and R. Schehr, *Exp. Neurol.*, 1997, **145**, 89–92.
23. D. C. Goff, J. S. Lamberti, A. C. Leon, M. F. Green, A. L. Miller, J. Patel, T. Manschreck, O. Freudenreich and S. A. Johnson, *Neuropsychopharmacology*, 2007, **33**, 465–472.
24. a) E. Ward and M. Harries, *Curr. Med. Chem.*, 2010, **17**, 3503–3513; b) S. J. A. Grove, C. Jamieson, J. K. F. Maclean, J. A. Morrow and Z. Rankovic, *J. Med. Chem.*, 2010, **53**, 7271–7279; c) J. A. Morrow, J. K. Maclean and C. Jamieson, *Curr. Opin. Drug Discov. Devel.*, 2006, **9**, 571–579.
25. A. S. Chappell, C. Gonzales, J. Williams, M. M. Witte, R. C. Mohs and R. Sperling, *Neurology*, 2007, **68**, 1008–1012.
26. O. P. Hamill, A. Marty, E. Neher, B. Sakmann and F. J. Sigworth, *Pflugers Arch.*, 1981, **391**, 85–100.
27. S. E. Ward, M. Harries, L. Aldegheri, N. E. Austin, S. Ballantine, E. Ballini, D. M. Bradley, B. D. Bax, B. P. Clarke, A. J. Harris, S. A. Harrison, R. A. Melarange, C. Mookherjee, J. Mosley, G. Dal Negro, B. Oliosi, K. J. Smith, K. M. Thewlis, P. M. Woollard and S. P. Yusaf, *J. Med. Chem.*, 2011, **54**, 78–94.

28. K. M. Thewlis, L. Aldegheri, M. H. Harries, C. Mookherjee, B. Oliosi and S. E. Ward, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7116–7119.
29. a) N. Armstrong and E. Gouaux, *Neuron*, 2000, **28**, 165–181; b) H. Furukawa, S. K. Singh, R. Mancusso and E. Gouaux, *Nature*, 2005, **438**, 185–192.
30. S. Bailey, *Acta Crystallogr. Sect. D: Biol. Crystallogr.*, 1994, **D50**, 760–763.
31. G. N. Murshudov, A. A. Vagin and E. J. Dodson, *Acta Crystallogr. Sect. D: Biol. Crystallogr.*, 1997, **53**, 240–255.
32. P. Emsley and K. Cowtan, *Acta Crystallogr. Sect. D: Biol. Crystallogr.*, 2004, **60**, 2126–2132.
33. J. Pøhlsgaard, K. Frydenvang, U. Madsen and J. S. Kastrop, *Neuropharmacology*, 2011, **60**, 135–150.
34. P. L. Ornstein, D. M. Zimmerman, M. B. Arnold, T. J. Bleisch, B. Cantrell, R. Simon, H. Zarrinmayeh, S. R. Baker, M. Gates, J. P. Tizzano, D. Bleakman, A. Mandelzys, K. R. Jarvie, K. Ho, M. Deverill and R. K. Kamboj, *J. Med. Chem.*, 2000, **43**, 4354–4358.
35. a) C. Sager, D. Tapken, S. Kott and M. Hollmann, *Neuroscience*, 2009, **158**, 45–54; b) J. Schwenk, N. Harmel, G. Zolles, W. Bildl, A. Kulik, B. Heimrich, O. Chisaka, P. Jonas, U. Schulte, B. Fakler and N. Klocker, *Science*, 2009, **323**, 1313–1319; c) Y. Shi, W. Lu, A. D. Milstein and R. A. Nicoll, *Neuron*, 2009, **62**, 633–640.
36. A. W. Kimball, W. T. Burnett, Jr and D. G. Doherty, *Radiat. Res.*, 1957, **7**, 1–12.
37. A. I. Sobolevsky, M. P. Rosconi and E. Gouaux, *Nature*, 2009, **462**, 745–756.
38. S. E. Ward, M. Harries, L. Aldegheri, D. Andreotti, S. Ballantine, B. D. Bax, A. J. Harris, A. J. Harker, J. Lund, R. Melarange, A. Mingardi, C. Mookherjee, J. Mosley, M. Neve, B. Oliosi, R. Profeta, K. J. Smith, P. W. Smith, S. Spada, K. M. Thewlis and S. P. Yusaf, *J. Med. Chem.*, 2010, **53**, 5801–5812.
39. R. Bursi, G. Erdemli, R. Campbell, M. M. Hutmacher, T. Kerbusch, D. Spanswick and R. Jeggo, K. R. Nations, P. Dogterom, J. Schipper and M. Shahid, *Psychopharmacology*, 2011, **218**(4), 713.

## CHAPTER 6

# *Treating the Cognitive Deficits of Schizophrenia*

BRUCE N. ROGERS\* AND DAVID L. GRAY

Worldwide Medicinal Chemistry – Neuroscience, Pfizer Global Research and Development, 700 Main Street, Cambridge, MA 02139, USA

\*Email: bruce.n.rogers@pfizer.com

## **6.1 Introduction: Biological Rationale for Treating the Cognitive Deficits in Schizophrenia**

Schizophrenia is a debilitating chronic psychiatric disorder that affects approximately 1% of the US population. The disorder presents in a constellation of symptoms that are commonly clustered into subsets known as positive, negative and cognitive. Since the 1990s, significant progress has been made in treatments for schizophrenia, and many of these approaches are covered elsewhere in this book. Even with the introduction of new therapies, however, evidence suggests that about two-thirds of patients are treated ineffectively by current medications.<sup>1</sup> The cognitive deficits in schizophrenia (CDS) are recognized as a discrete cluster of impairments that, left untreated, result in poor functional outcomes in work and social and independent living. Although existing antipsychotic drugs effectively treat positive symptoms, they provide inadequate improvement in CDS in most schizophrenia patients and thus have a limited effect on the course of this chronic illness. A team of experts across industry and academia was assembled in the early 2000s by the National Institute of Mental Health (NIMH) to respond to emerging scientific advances

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)



**Table 6.1** Top MATRICS initiative targets in order of importance.

<i>Target</i>	<i>Ranking</i>
$\alpha 7$ Nicotinic acetylcholine receptor agonist ( $\alpha 7$ nAChR)	1
Dopamine D <sub>1</sub> receptor agonists	2
$\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) glutamatergic receptor agonists	3
$\alpha 2$ Adrenergic receptor agonists	4
<i>N</i> -methyl-D-aspartate (NMDA) glutamatergic receptor agonists	5
Metabotropic glutamate receptor agonists (mGluR)	6
Glycine reuptake inhibitors (GlyT)	7
M <sub>1</sub> muscarinic receptor agonists (M <sub>1</sub> mAChR)	8
$\gamma$ -Aminobutyric acid A receptor subtype selective agonists (GABA <sub>A</sub> )	9

in the cognitive neuropsychology neurobiology of schizophrenia.<sup>2</sup> Research through a National Institute of Mental Health project called Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) clearly raised awareness and formed consensus among experts in the field that most patients with schizophrenia experience CDS and that novel treatment options need to be developed to address it.<sup>3</sup> Since then, a notable push to develop treatments for CDS has occurred, but significant challenges persist. In addition to raising awareness and developing clinical outcome measures, the MATRICS team defined the top nine targets for CDS treatments (Table 6.1). To reach consensus on the targets, experts in the areas represented by each target presented supporting arguments for its the inclusion on the list. Through discussion and debate, the two most promising mechanisms identified were  $\alpha 7$  nicotinic acetylcholine (ACh) receptor (nAChR) agonists and dopamine 1 (D<sub>1</sub>) receptor agonists. This chapter focuses on the chemical landscape and medicinal chemistry challenges of the mechanisms being actively researched, focusing primarily on these two high-priority targets recommended by the MATRICS team. In addition, brief updates on the current state of other possible CDS treatments, such as M<sub>1</sub> muscarinic ACh receptor (mAChR) agonists and positive allosteric modulators (PAMs), serotonin subtype 6 receptor (5-HT<sub>6</sub>) antagonists and histamine 3 (H<sub>3</sub>) antagonists, which are the focus for CDS treatments, are given. The glutamatergic approaches of glycine transporters and  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid are also of significant interest for the potential treatment of CDS, and they are reviewed extensively in Chapters 4 and 5.

## 6.2 Alpha 7 Nicotinic Acetylcholine Receptors

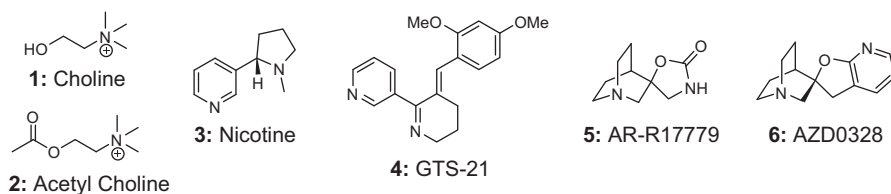
### 6.2.1 Alpha 7 Nicotinic Acetylcholine Receptor Agonists

Neuronal nAChRs are believed to be involved in a variety of attention and cognitive processes.<sup>4</sup> These Ca<sup>+2</sup>-permeable, ligand-gated ion channels modulate synaptic transmission in key regions of the brain involved in learning and

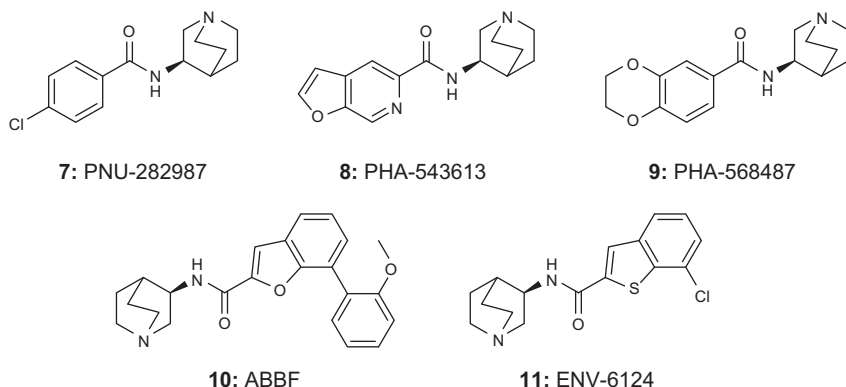
memory, including the hippocampus, thalamus and cerebral cortex.<sup>5–7</sup> Among the nAChRs, physiological, pharmacological and human genetic data suggest a link between the loss of  $\alpha 7$  nAChRs and sensory gating deficits in schizophrenia.<sup>8</sup> Conversely, improvements in sensory processing are thought to correlate with enhanced cognitive performance in animal models and patients with schizophrenia,<sup>9</sup> suggesting a role for selective  $\alpha 7$  nAChR agonists in the treatment of CDS. The activation of the  $\alpha 7$  nAChR by its endogenous agonist ACh (**2**) has been studied in great detail, particularly the molecular-level interactions between agonists and the receptor-binding sites of the receptor.<sup>10–12</sup> In addition to the well-established endogenous agonist ACh, choline (**1**),<sup>13</sup> a precursor and metabolite of ACh, is also considered an endogenous agonist of  $\alpha 7$  nAChRs with kinetic properties of activation and desensitization similar to those of ACh.<sup>14</sup> ACh (**2**) and choline (**1**; Figure 6.1) as well as the nAChR natural ligand nicotine (**3**) are small-molecular-weight molecules that are highly efficient activators of the receptor. The inherent challenge with these ligands lies in their lack of selectivity for the  $\alpha 7$  nAChR over other nAChRs, and thus they are of limited utility for the development of a full understanding of  $\alpha 7$  nAChR subtype pharmacology.

Since 2000, impressive preclinical effort has been invested in the identification of agonists of  $\alpha 7$  nAChRs, and the medicinal chemistry aspects have been exhaustively reviewed.<sup>15–27</sup> This focus has provided solutions for significant medicinal chemistry challenges and yielded numerous novel and selective agents that have entered clinical trials. Many of the ligands described in the primary literature as  $\alpha 7$  nAChR agonists are derived from the quinuclidine scaffold (**5–20**, Figures 6.1–6.2) and include such structures as spirooxazolidinone and quinuclidine carbamates. Several other templates have appeared, expanding the field to include additional azabicyclic amine templates and, in a significant departure from the quinuclidine template, the anabasine analogues of GTS-21 (3-2,4 dimethoxybenzylidene anabasine [DMXB-A] **4**; see Figure 6.1). The discussion in this section focuses on compounds that have entered clinical studies.

The anabasine analogue GTS-21 (**4**) was one of the earliest reported non-nicotine ligands for the  $\alpha 7$  nAChR and is believed to be a functionally selective partial agonist ( $\alpha 7$  nAChR half maximal effective concentration [ $EC_{50}$ ] = 81  $\mu$ M).<sup>28,29</sup> One of the most significant challenges of the anabasine series has been binding selectivity, and GTS-21 (**4**) is a potent binder to the  $\alpha 4\beta 2$  nAChR



**Figure 6.1** Structures of  $\alpha 7$  nicotinic acetylcholine receptor agonists.



**Figure 6.2** Structures of  $\alpha 7$  nicotinic acetylcholine receptor agonists.

subtype with an affinity on the order of 100-fold greater than that of its  $\alpha 7$  binding.<sup>30</sup> GTS-21 has been extensively characterized *in vitro* and *in vivo* and is also one of a handful of compounds that has advanced to clinical trials, where it initially showed statistically significant enhancement of attention, working memory and episodic secondary memory in healthy male subjects<sup>31</sup> and improved cognition in non-smoking patients currently taking antipsychotic medications.<sup>32</sup> A larger phase II study in 31 patients with schizophrenia treated for 4 weeks (75 mg bid or 150 mg bid po)<sup>33</sup> found no significant improvement in primary cognition (MATRICS cognition battery). During the study a significantly higher incidence of nausea was seen in patients treated with a higher dose, suggesting that compounds that contain both  $\alpha 7$  activity and serotonin subtype 3 receptor (5-HT<sub>3</sub>) antagonism could be useful agents.<sup>34</sup> In addition, the high doses necessary to produce an effect suggest that a more potent compound would be desirable, a property that has been challenging to attain within this series. Although the clinical results have fallen short of the original hypothesis of the study, they are encouraging, particularly given the limitations of DMXB-A.

The most significant of the investigated ligands of the  $\alpha 7$  nAChR are the quinuclidine amine-based compounds. Among the first compounds from this group were the spirooxazolidinones, which were developed by researchers at AstraZeneca.<sup>35</sup> Initially characterized by AR-R17779 (**5**; see Figure 6.1), this class has evolved through structure-activity relationship (SAR) optimization by several research groups to an active, selective *in vivo* series of compounds. Although a great small-molecule tool, the initial compound also activated 5-HT<sub>3</sub> receptors and had limited brain penetration. The spirooxazolidinones have been further refined by researchers at several companies,<sup>36</sup> and AstraZeneca has identified AZD0328 (**6**; see Figure 6.1) as a clinical candidate that has overcome the issues of 5-HT<sub>3</sub> activation and poor brain penetration.<sup>37</sup> This potent  $\alpha 7$  nAChR agonist ( $K_i = 3$  nM;  $EC_{50} = 338$  nM) has been described as a selective partial agonist with PK characteristics adequate enough to progress

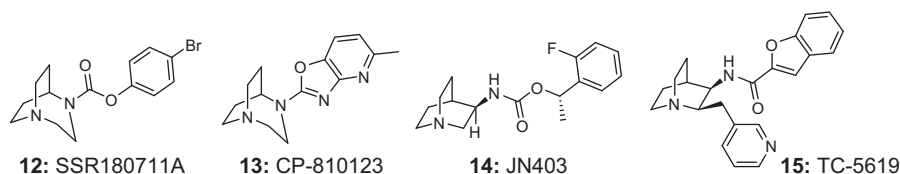
to human studies<sup>38</sup> that demonstrated *in vivo* activity in a variety of models including mouse novel object recognition, reversal of short-term memory deficits in fimbria-fornix-lesioned rats and improvement in working memory in rhesus monkey spatial delayed-response performance.<sup>39</sup> A recent publication on AZD0328 has suggested the possibility that very low doses (0.00178 mg/kg) of this  $\alpha 7$  nAChR agonist can improve cognition in preclinical models.<sup>40</sup> In addition to this finding, studies by other investigators cited throughout the remainder of this section imply a potential for clinical efficacy in this mechanism at very low levels of receptor occupancy.

Many of the ligands disclosed in the literature that have entered clinical studies come from the quinuclidine scaffold.<sup>41–44</sup> The most extensively characterized ligands in this area are the quinuclidine amides and carbamates. A key compound that emerged early in the literature is the quinuclidine benzamide PNU-282987 (**7**; Figure 6.2), developed by researchers at Pharmacia/Pfizer.<sup>45</sup> PNU-282987 (**7**) is a potent and selective  $\alpha 7$  nAChR agonist ( $K_i = 29$  nM), with full efficacy relative to nicotine in functional assays. An important characteristic of this compound was its significant activity at the human Ether-a-Go-go Related Gene (hERG) channel. This molecule restores P50 gating deficits in rodents after oral dosing.<sup>46</sup> The compound has been characterized extensively *in vitro* and *in vivo* and has served as an excellent tool to guide research efforts. Researchers at Pharmacia/Pfizer have also described a series of agonist compounds – best exemplified by PHA-543613 (**8**) and PHA-568487 (**9**; see Figure 6.2) – that have profiles similar to that of PNU-282987 with significantly reduced hERG interaction and improved absorption, distribution, metabolism and excretion properties.<sup>47,48</sup> PHA-543613 (**8**) and PHA-568487 (**9**) demonstrated sufficient preclinical efficacy and safety to support their entry into phase I single ascending dose and multiple ascending dose safety, tolerability and pharmacokinetic (PK) studies. PHA-543613 (**8**) also demonstrated a linear PK profile and a desirable half-life in humans ( $\sim 9$ – $12$  h).<sup>49</sup> In the CogState cognitive testing battery, subjects showed a modest improvement in cognitive function at a low dose (6 mg bid) but not at higher doses. A low frequency of cardiovascular arrhythmia with no evidence of QTc prolongation or other electrocardiographic interval changes was also observed during these studies. As a result, clinical development of both PHA-543613 (**8**) and PHA-568487 (**9**) was discontinued owing to a low incidence (5%) of asymptomatic non-sustained ventricular tachycardia and premature ventricular contraction in healthy volunteers.<sup>50</sup> In a recent analysis, the receptor occupancy measured for PHA-543613 (**8**) was projected to be  $> 75\%$  for the low dose (6 mg) and  $> 94\%$  for the higher doses tested in the multi-dose phase of the study.<sup>51</sup> As with other neuronal ion channels, prolonged exposure of the  $\alpha 7$  nAChR to an agonist can cause desensitization, a phenomenon through which continuous agonist-mediated receptor stimulation leads to receptor inactivation. From a medicinal chemistry perspective, this characteristic should be considered carefully, as too great an exposure over a prolonged period could result in a loss of desired agonistic activity and the development of antagonist-like activity. From a receptor occupancy standpoint, the clinical doses of PHA-543613 (**8**) evaluated

in the multi-dose study suggest that lower doses (*i.e.*  $\leq 6$  mg bid) may have been optimal for cognition trials in subjects with schizophrenia and that higher doses were not beneficial.

The quinuclidine amide ABBF (**10**; see Figure 6.2) has been reported by researchers by Bayer to be a full agonist at  $\alpha 7$  nAChRs (negative logarithm  $EC_{50}$  [ $pEC_{50}$ ] = 5.5), with weak antagonist activity at other nAChRs.<sup>52</sup> Like the Pharmacia series, ABBF (**10**) showed antagonism at the 5-HT<sub>3</sub> receptor. ABBF also demonstrated *in vivo* activity when dosed orally (0.3 and 1.0 mg/kg) in a variety of models including rat social recognition, mouse novel object recognition and rat water maze. Using this series of compounds, researchers at EnVivo Pharmaceuticals reported that (*R*)-7-chloro-*N*-(quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide (EVP-6124 [**11**]; see Figure 6.2) is a potent and selective  $\alpha 7$  nAChR agonist (human  $\alpha 7$  nAChR  $EC_{50}$  = 158 nM; rat  $\alpha 7$  nAChR  $K_i$  = 12 nM) that produces procognitive effects in humans.<sup>53</sup> EVP-6124 (**11**) increases P300 response at doses of 0.3 and 1.0 mg. The EnVivo team also reported that healthy human volunteers using the CogState battery in the multi-dose phase of clinical trials demonstrated procognitive effects at daily doses of EVP-6124 (**11**) as low as 1 mg. Although the full details of these studies have yet to be disclosed, these data suggest that very low levels of free drug concentration – concentrations below the  $\alpha 7$  nAChR  $K_i$  of the compound, in fact – are required to improve cognition. This finding of efficacy at low dose is consistent with both the findings of the AstraZeneca group and the hypothesis put forward earlier in this section that low levels of receptor occupancy are required for effects through the  $\alpha 7$  nAChR agonist mechanism.

Reports describing additional structural diversity in the amine or linker portions of  $\alpha 7$  nAChR ligands have also appeared in the primary literature (Figure 6.3). Significant characterization of alternate amines has been published for the diazabicyclononanes SSR180711A (**12**)<sup>54,55</sup> and CP-810123(**13**),<sup>56</sup> the quinuclidine carbamate JN403 (**14**) and the substituted quinuclidine TC-5619 (**15**).<sup>57</sup> The selective partial agonist SSR180711A (**12**) is a 1,4-diazabicyclo[3.2.2]nonane carbamate derivative ( $K_i$  = 50 nM;  $EC_{50}$  = 800 nM) active in novel object recognition, Morris water maze and MK-801-induced memory deficit models. Although numerous publications on this compound have appeared, whether it progressed into clinical development is uncertain. A research group at Pfizer has published on the chloro analogue of SSR180711A (**12**),<sup>58</sup> which led to the discovery of CP-810123 (**13**). CP-810123 (**13**) exhibits an excellent balance of potency ( $K_i$  = 13.3 nM), selectivity (5-HT<sub>3</sub>  $K_i$  = 244 nM



**Figure 6.3** Structures of  $\alpha 7$  nicotinic acetylcholine receptor agonists.

as an antagonist) and high-affinity agonist activity at the  $\alpha 7$  nAChR ( $EC_{50}$  = 244 nM in oocytes; 195% efficacy *vs.* nicotine) and represents a novel chemotype. The *in vitro* profile was complemented by excellent brain penetration, high oral bioavailability and *in vivo* activity in two models of cognition, which prompted clinical studies.<sup>56</sup> Phase I single- and multi-dose studies evaluating the safety, tolerability and PK characteristics of CP-810123 demonstrated a linear PK profile and a half-life in humans comparable to those of PHA-543613 and PHA-568487.<sup>51</sup> On the CogState battery, subjects taking CP-810123 demonstrated a modest improvement in cognitive function at the lowest dose tested (5 mg); however, they also reported episodes of non-sustained ventricular tachycardia at high doses and, in light of the clinical data on PHA-543613 and PHA-568487, development of CP-810123 was also halted.<sup>51</sup>

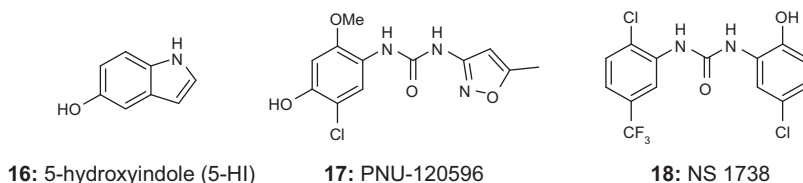
The substituted quiniclidine TC-5619 (**15**) represents an additional modification to the quiniclidine ring structure (see Figure 6.3). TC-5619 (**15**) has 1 nM affinity for the  $\alpha 7$  nAChR and selectivity for the other nicotinic receptors, and it represents a potent full agonist, as measured in an oocyte assay ( $EC_{50}$  = 33 nM; 100% agonist relative to ACh).<sup>59</sup> The *in vitro* and *in vivo* profiles of this compound were compelling enough for Targacept to bring it into clinical studies. The company has reported positive clinical outcomes on measures of cognition and negative symptoms in schizophrenia.<sup>60</sup> Investigators at Novartis recently disclosed an entry in the quinuclidine area containing a carbamate linker as JN403 (**14**; see Figure 6.3).<sup>61</sup> The *in vitro* properties of this compound demonstrate that it is a potent and selective  $\alpha 7$  nAChR ( $pK_D$  = 6.7) and a partial agonist relative to ACh in *Xenopus* oocytes. The *in vivo* characteristics of this compound have also been reported: it demonstrates robust PK characteristics with rapid brain penetration and activity in rat social exploration, mouse sensory gating (dilute brown, non-agouti mice) and two models of permanent pain.<sup>62</sup>

### 6.2.2 Alpha 7 Nicotinic Acetylcholine Receptor Positive Allosteric Modulators

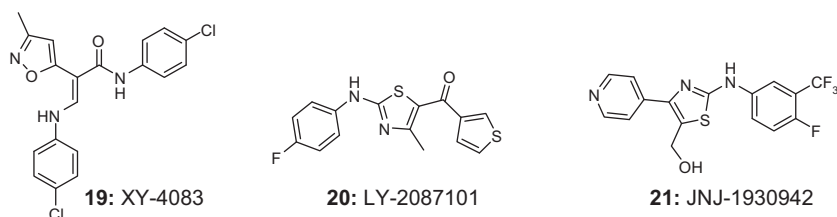
An alternative method for activating the  $\alpha 7$  nAChR is the harnessing of the endogenous ligand ACh in concert with a positive allosteric modulator (PAM). This approach provides a synergistic augmentation of the natural ligand and has the potential to avoid undesired receptor desensitization.<sup>63</sup> Interest in allosteric modulators of the  $\alpha 7$  nAChR in medicinal chemistry has expanded in recent years and focuses on the identification of novel chemical entities to build biological understanding. This section focuses on the allosteric ligands that have been most extensively characterized in the primary literature and have thus served as tools that have significantly advanced the field overall.

Early efforts to demonstrate the benefit of allosteric modulators of the  $\alpha 7$  nAChR used 5-hydroxy indole (**16**; Figure 6.4), but its lack of selectivity and potency led instead to the identification of other ligands.<sup>64</sup> The disclosure of the  $\alpha 7$  nAChR PAM PNU-120596 (**17**) has provided a selective tool for further





**Figure 6.4** Structures of  $\alpha 7$  nicotinic acetylcholine receptor positive allosteric modulators.



**Figure 6.5** Structures of  $\alpha 7$  nicotinic acetylcholine receptor positive allosteric modulators.

exploration of the PAM mechanism.<sup>65</sup> PNU-120596 (**17**) increases agonist-evoked  $\text{Ca}^{+2}$  flux mediated by an engineered variant of the human  $\alpha 7$  nAChR and enhances ACh-evoked inward currents in hippocampal interneurons. This compound has also suppressed desensitization when tested *in vitro* and has shown robust *in vivo* activity in an amphetamine-induced P50 gating deficit model (0.1–3 mg/kg iv). Although a narrow SAR has been described, the key challenges of this series from a medicinal chemistry perspective have been to improve potency, physiochemical properties and PK characteristics, and key *in vivo* improvements have been identified.<sup>66,67</sup> PNU-120596 (**17**) has demonstrated activity in many of the same preclinical models as those with selective  $\alpha 7$  NACHR agonist activity, indicating strong potential for this class of compounds.

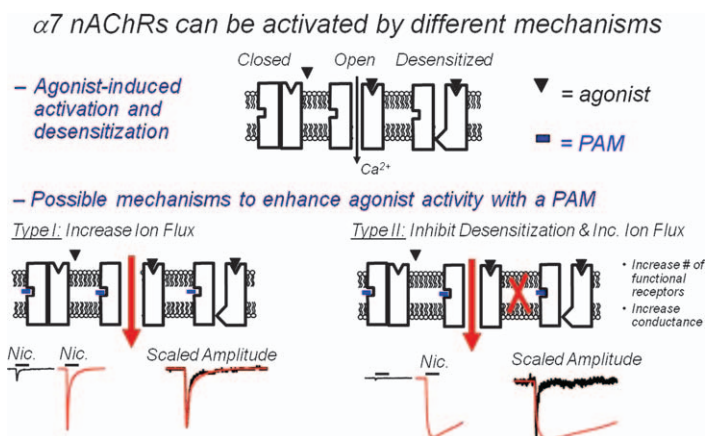
A biaryl urea series exemplified by NS1738 (**18**) has also appeared in the literature, originating from researchers at NeuroSearch.<sup>68</sup> This compound has also been reported to enhance the potency and efficacy of an agonist, but it does not appear to affect desensitization to the extent that PNU-120596 (**17**) does. Although NS1738 (**18**) has only modest brain penetration, it was able to rescue a (–)-scopolamine-induced deficit in the acquisition of a water-maze learning task in rats and improve performance in rat social recognition *in vivo*.

Researchers at the University of California, Irvine, recently made and exploited an interesting connection between the  $\gamma$ -aminobutyric acid A (GABA<sub>A</sub>) receptor and the  $\alpha 7$  nAChR based on sequence homology<sup>69</sup> to identify a library of modulators of GABA<sub>A</sub> and, through a series of SAR manipulations, XY4083 (**19**; Figure 6.5).<sup>70</sup> XY4083 evokes positive modulation



of agonist-induced currents, which were demonstrated to retain the kinetic and desensitization properties of the  $\alpha 7$  nAChR. This compound also reverses sensory gating deficits in rodents and improves working memory *in vivo*. Eli Lilly and Johnson & Johnson (JNJ) have independently reported that high-throughput screening (HTS) campaigns yielded thiazole leads that act as  $\alpha 7$  nAChR PAMs. LY-2087101 (**20**) appears to be selective for the brain forms of the nAChR but not specifically for the  $\alpha 7$  nAChR subtype. Further profiling of these compounds has demonstrated that they indeed enhance potency and maximal efficacy at both the  $\alpha 7$  and the  $\alpha 4\beta 2$  nAChRs.<sup>71</sup> More recently, the group at JNJ has disclosed JNJ-1930942 (**21**), also a thiazole, stating it to be selective for the  $\alpha 7$  nAChR.<sup>72</sup> This compound increases peak current amplitude and, like PNU-120596 (**17**), appears to slow desensitization kinetics and affect auditory evoked potentials in dilute brown, non-agouti mice. Notably, subtle differences in this class appear to have profound effects on selectivity for the neuronal nAChR subtypes.

As literature reports on PAMs for the  $\alpha 7$  nAChR expand, it is growing clear that they have at least two modes of action (Figure 6.6).<sup>73</sup> Studies with homomeric  $\alpha 7$  nAChRs indicate that such ligand interactions can be well described by an allosteric model<sup>74</sup> and that positive allosteric effectors can modify energy transitions by (1) predominantly affecting peak current response (type I profile) or (2) increasing both peak current response and the desensitization profile of an agonist (type II profile).<sup>75</sup> The recent literature described above discloses a chemically diverse group of molecules capable of differentially modifying nAChR properties without interacting at the ligand-binding site. Compounds that clearly appear to fit the type I mechanism – 5-HI (**16**), XY-4083 (**19**) and NS1738 (**18**) – have little or no effect on desensitization kinetics *in vitro*. Compounds that fit the type II mechanism – PNU-120596 (**17**), LY-2087101 (**20**) and JNJ-1930942 (**21**) – affect the desensitization kinetics to



**Figure 6.6** Activation pathways for type I and II nicotinic acetylcholine receptor (nAChR) positive allosteric modulators (PAMs).

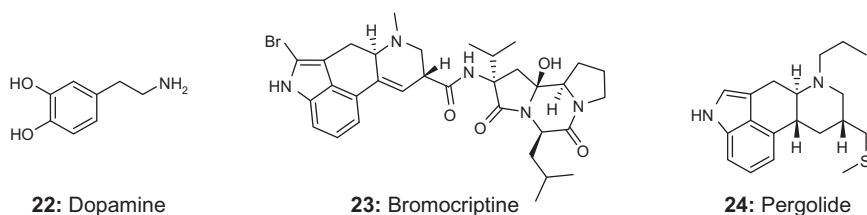
differing degrees, with PNU-120596 (**17**) producing the most profound effect *in vitro*. Notably, both modes of allosteric activation produce similar effects *in vivo*. A key SAR challenge that remains to be addressed within this mechanism is the determination of which profile will be acceptable in the clinical setting.

The investment of medicinal chemistry efforts to identify an activator of the  $\alpha 7$  nAChR through an agonist or PAM has been substantial since the mid 1990s, and it clearly offers significant opportunity given its selection by the MATRICS initiative as the mechanism with the highest priority. Numerous medicinal chemistry challenges have been overcome to produce compounds that have entered clinical trials from the agonist arena. Similarly, the availability of tool compounds has precipitated a much deeper understanding of the mechanisms through which  $\alpha 7$  nAChR PAMs act. Given such clear progress, this top-priority mechanism will be definitively tested in proof-of-concept trials to advance understanding of this target in the treatment of CDS.

### 6.3 Dopamine D<sub>1</sub> Receptor Agonists

The second mechanism identified by the MATRICS initiative to be of significant interest in the treatment of CDS is agonism of the D<sub>1</sub> receptor. Since the discovery of this subtype in 1979, medicinal chemists in academia and industry have been targeting selective D<sub>1</sub> agonists.<sup>76</sup> Although several long-running discovery programs have succeeded in advancing selective D<sub>1</sub> agonists into clinical development, each of the compounds profiled to date contains a catecholamine functionality associated with poor PK characteristics and impaired brain penetration.<sup>77–79</sup> The emergence of preclinical data (briefly described at the end of this chapter) suggesting that agonism of D<sub>1</sub> would have therapeutic utility in treating CDS post-dates the termination of several major D<sub>1</sub> agonist preclinical discovery programs.<sup>80,81</sup>

Dopamine (**22**) is a main neurotransmitter, and research into its action can be traced to the early days of neuroscience (Figure 6.7). In the 1950s, a serendipitous finding that certain sedatives significantly reduced many of the overt manifestations of schizophrenia revolutionized the treatment of the illness.<sup>82</sup> In the 1960s, seminal work by Carlsson and others revealed that dopamine antagonism was the common pharmacology of effective antipsychotic agents, highlighting the importance of dopaminergic pathways in schizophrenia.<sup>83,84</sup>



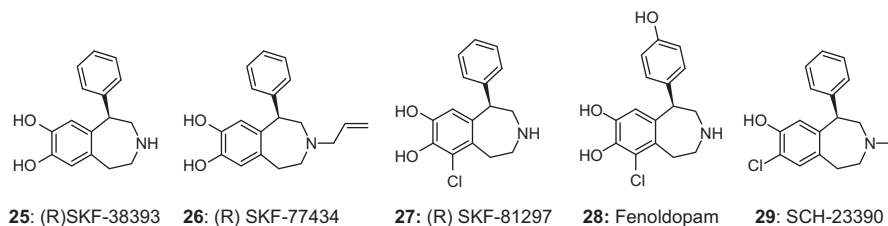
**Figure 6.7** Structures of dopamine agonists.

Investigators later established a direct relationship between the degree of occupancy of the dopamine D<sub>2</sub> receptor in the brain and the efficacy of a compound in schizophrenia.<sup>85</sup> Through clinical and preclinical observations, the “dopamine hypothesis” of schizophrenia began to emerge and gain traction.<sup>86</sup> The original hypothesis postulated that excessive activation of striatal dopamine receptors was responsible for many of the positive symptoms of schizophrenia. In the late 1970s, Keabian<sup>87</sup> demonstrated that two major classes of dopamine receptors were present – those that were positively coupled to adenylate cyclase (D<sub>1</sub>-like, including D<sub>1</sub> and D<sub>5</sub>) and those that were negatively coupled (D<sub>2</sub>-like, including D<sub>2</sub>, D<sub>3</sub> and D<sub>4</sub>). With heavy reliance on behavioural disease models in rodents, empirical observations suggested that agonizing D<sub>1</sub>-like receptors might lead to antihypertensive or anti-Parkinsonian agents.<sup>88</sup> Several groups of researchers in industry and academia began medicinal chemistry efforts targeting selective D<sub>1</sub> agonist compounds for these indications.<sup>89</sup>

Frequently (including in this chapter), the D<sub>1</sub> and D<sub>5</sub> subtypes are bundled together and referred to as D<sub>1</sub>-like or simply D<sub>1</sub>. To date, no small-molecule agonists with significant and validated selectivity for D<sub>1</sub> *versus* D<sub>5</sub> have been disclosed in the literature. Although a few reports of D<sub>5</sub>-selective antagonists have appeared, none has been embraced or applied widely as a preclinical tool.<sup>90</sup> Apart from investigation into the phenotypes of D<sub>1</sub> or D<sub>5</sub> knockout mice, few *in vivo* data are available to distinguish the functional activation of D<sub>1</sub> from that of D<sub>5</sub> and, at present, researchers have largely resigned themselves to describing actions at the two subtypes collectively as occurring *via* D<sub>1</sub>.<sup>91</sup> Medicinal chemistry programs focusing on early D<sub>1</sub> agonists started either by making analogues of dopamine itself or by modifying known non-selective dopamine agonist lead compounds, including the important class of ergot alkaloids that were initially discovered from natural product screens. Through heroic synthetic and semi-synthetic modification, several benzergoline-based dopamine agonist drugs, including bromocriptine (**23**; hD<sub>1</sub> K<sub>i</sub> = 1,500 nM; hD<sub>2</sub> K<sub>i</sub> = 12 nM) in 1975 and pergolide (**24**; hD<sub>1</sub> K<sub>i</sub> = 310 nM; hD<sub>2</sub> K<sub>i</sub> = 33 nM) in 1995 (see Figure 6.7) were brought to market for the treatment of Parkinson’s disease.<sup>92–96</sup> In general, the benzergolines are potent agonists at all dopamine subtypes, with particularly high affinity for D<sub>2</sub>-like receptors. Despite significant medicinal chemistry effort dedicated to this non-catechol scaffold, SAR studies never advanced to produce D<sub>1</sub>-selective compounds.<sup>95</sup>

The first truly selective D<sub>1</sub> agonists were disclosed by researchers at Smith, Kline & French (SKF) in the late 1970s. To achieve selectivity, they constrained dopamine within a larger polycyclic benzazepine framework, which yielded the active leads shown in Figure 6.8, including the much-studied SKF-38393 (**25**; human D<sub>1</sub> K<sub>i</sub> = 18 nM; human D<sub>2</sub> K<sub>i</sub> = 9,300 nM), SKF-77434 (**26**; hD<sub>1</sub> K<sub>i</sub> = 11 nM; hD<sub>2</sub> K<sub>i</sub> = 1,000 nM) and, later, SKF-81297 (**27**; hD<sub>1</sub> K<sub>i</sub> = 32 nM; hD<sub>2</sub> K<sub>i</sub> = 1,270 nM) within a common 3-phenyl benzazepine structure.<sup>97–101</sup>

Most of these compounds were profiled as racemates; however, one enantiomer was typically shown to have much more affinity for the D<sub>1</sub> receptor than the other.<sup>102</sup> The synthesis of these molecules was challenging but, over several

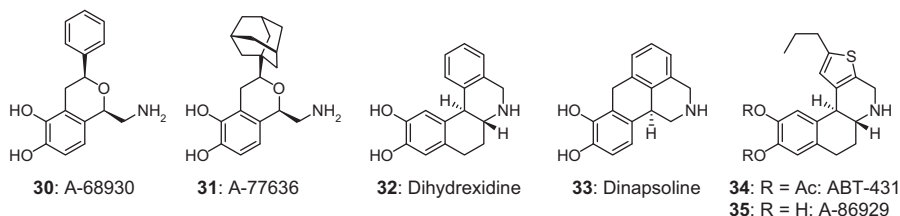


**Figure 6.8** Structures of selective catechol dopamine  $D_1$  agonists related to antagonist SCH-23390.

years, a number of analogues with improved potency, varied intrinsic functional efficacy at  $D_1$  and improved selectivity *versus*  $D_2$  receptors were disclosed. In 1998, nearly 20 years after the initial disclosure of the benzazepine scaffold, GlaxoSmithKline (GSK) launched a compound from this series as the peripherally restricted antihypertensive fenoldopam (**28**), sold in the USA as intravenously administered Corlopan ( $hD_1 K_i = 3 \text{ nM}$ ;  $hD_2 K_i = 1,580 \text{ nM}$ ; see Figure 6.8).<sup>103–107</sup> The benzylic phenyl moiety is a major contributor to binding potency and imparts significant selectivity *versus*  $D_2$ -like receptors. Of the benzazepines, SKF-38393 (**25**;  $hD_1 E_{\max} = 50\%$  relative to dopamine) and SKF-77434 (**26**;  $hD_1 E_{\max} = 25\%$  relative to dopamine) were determined to be partial agonists at  $D_1$ , whereas close-in analogues such as SKF-81297 (**27**) showed full agonist activity.<sup>108–112</sup> The therapeutic consequence of partial *versus* full agonism of  $D_1$ -like receptors remains an open question, although many published reports have suggested that compounds with varied intrinsic agonist activities have differential effects in various behavioural models and *in vitro* systems.<sup>101,113,114</sup>

The catechol functionality and the basic amine were both deemed necessary for driving potency and  $D_1$  agonist activity within this scaffold, although it is possible to substitute on the amine with small alkyl chains or make other minor modifications with no loss of potency.<sup>97,98,115–117</sup> These functionalities have presented a significant challenge, however, as potential clinical agents from this class have been associated with short circulating half-lives and minimal oral bioavailability, presumably resulting from rapid phase II metabolism directed at the catechol moiety *in vivo*.<sup>79,118</sup> Building on the leads reported by the SKF group, investigators at Schering-Plough produced a structurally related class of  $D_1$  antagonists. An antagonist first disclosed in 1987 as, SCH-23390 (**29**;  $hD_1 K_i = 1.4 \text{ nM}$ ;  $hD_2 K_i > 1,000 \text{ nM}$ ; see Figure 6.8), and now used clinically as a ( $^{11}\text{C}$ )  $D_1$  PET ligand is a close analogue of SKF-38393 (**25**) wherein one phenolic group is replaced by a chloride, and the amine is methylated.<sup>119,120</sup> The subtle structural changes that impart agonism, partial agonism or antagonism within the phenyl benzazepine series of  $D_1$  agonists has been a widely discussed and debated subject in the literature.<sup>101,114</sup>

A successful  $D_1$  agonist research program at Abbott Laboratories examined several lead structures and introduced constrained dopamine analogues on a chiral amino benzopyran scaffold that were potent and selective  $D_1$  agonists



**Figure 6.9** Structures of selective catechol dopamine 1 agonists.

(Figure 6.9). The prototypical agent of this type was A-68930 (**30**;  $hD_1$   $K_i = 1$  nM;  $hD_2$   $K_i = >1,000$  nM).<sup>121</sup> The orientations of the amino group and the phenyl were deemed important for  $D_1$  binding, and other stereoisomers of this compound were less potent. A further refinement on this scaffold led to A-77636 (**31**;  $hD_1$   $K_i = 21$  nM;  $hD_2$   $K_i = >1,000$  nM), in which the phenyl replaced the extremely bulky adamantyl moiety was replaced.<sup>122,123</sup> This compound has been used as a tool  $D_1$  agonist in numerous studies and has been shown to have unusual slow-on/slow-off  $D_1$  binding kinetics.<sup>114</sup> In contrast to the benzazepines, which have modest affinity for multiple non-dopaminergic central nervous system (CNS) receptors, the Abbott structures are highly selective for  $D_1$  and  $D_5$ . Researchers at Abbott arrived at a similar conclusion regarding the necessity of the problematic catechol functionality, observing that both the catechol and the amine components were required for potency and functional activity. Although the benzopyrans represented an advance over the benzazepines in terms of potency and selectivity, they retained the catechol and thus demonstrated many of the PK challenges of compounds of this kind.<sup>78</sup>

Academic researchers at Purdue led by David Nichols designed another series of potent and selective  $D_1/D_5$  agonists, disclosing dihydropyridine (**32**;  $hD_1$   $K_i = 33$  nM;  $hD_2$   $K_i = 1,500$  nM) in 1989<sup>69</sup> and later dinapsoline (**33**;  $hD_1$   $K_i = 1.4$  nM;  $hD_2$   $K_i = 56$  nM; see Figure 6.9).<sup>124</sup> The dihydropyridine scaffold contains dopamine constrained into an active conformation within a tetracyclic framework. In agreement with previous findings, the stereochemistry of these molecules was important for proper orientation of the catechol, the amine and the bulky hydrophobic pharmacophore elements. Despite the structural differences between the Abbott, dihydropyridine and SKF cores, the pharmacophoric agreement between the three series is clear and suggests that optimal  $D_1$  functional activity is achieved by the constraint of dopamine into an extended conformation, whereas selectivity *versus*  $D_2$ -like receptors requires a bulky substituent with a defined projection relative to the amine and the catechol.<sup>125</sup> Both dihydropyridine and dinapsoline have been studied extensively as potential treatments for Parkinson's disease, with dihydropyridine having entered clinical studies.<sup>79</sup> In a continued search for leads with improved pharmacokinetics, both the Abbott  $D_1$  agonist and the Schering-Plough  $D_1$  antagonist programs moved to optimize the dihydropyridine-type rigid tetracyclic compounds (see Figure 6.9). A-82969 (**35**;  $hD_1$   $K_i = 49$  nM;  $hD_2$   $K_i = 710$  nM) is a close analogue of dihydropyridine and was the last clinical  $D_1$  agonist compound to

emerge from the long-running Abbott research effort.<sup>121,126</sup> Abbott used both pro-drug and novel delivery strategies to circumvent the first-pass metabolism liability of the catechol. A-86929 was the subject of a number of clinical formulation studies intended to advance it as a bis-acetyl inhaled pro-drug **34** known as ABT-431 (see Figure 6.9).<sup>121,127</sup> This latter derivative was dosed intravenously, orally and *via* inhalation and its effects characterized in multiple clinical trials, including a study in illicit drug users and two published trials in Parkinson's patients in whom it was efficacious. This compound had dose-limiting hypotensive effects, which may have contributed to its discontinuation from further development.<sup>78</sup>

The medicinal chemistry within the SKF, Abbott and dihydrexidine series of D<sub>1</sub> agonists was largely developed before the widespread use of rigorous methods for quantifying the extent of brain penetration for CNS compounds.<sup>128</sup> Like dopamine itself, catechol-based D<sub>1</sub> agonists from this era have multiple hydrogen-bond donors and frequently have impaired partitioning into the brain. The therapeutic effect of these compounds in Parkinson's or CDS is expected to derive *via* interaction with D<sub>1</sub> receptors located in the CNS. D<sub>1</sub> agonists with poor brain penetration have an increased likelihood of eliciting peripheral D<sub>1</sub>-driven, dose-limiting hypotensive effects in the clinic.<sup>79</sup> Medicinal chemists working in these programs began to appreciate the significant PK liabilities of the catechol-containing ligands and looked to design functional replacements; however, such changes frequently accompanied functional inefficacy. The identification of a non-catechol D<sub>1</sub> agonist or an allosteric approach to this target will be necessary to advance this treatment mechanism.

In parallel with (and aided by) the development of catechol-containing ligands, the dopamine hypothesis of schizophrenia was expanded to include a potential dopamine-centred explanation for CDS. Researchers in the Goldman-Rakic<sup>80</sup> laboratory and others convincingly demonstrated the critical role of D<sub>1</sub> receptor signalling in the prefrontal cortex during healthy cognition. Their work led to the hypothesis that hypodopaminergic signalling in the prefrontal cortex was responsible for the cognitive and working memory deficits observed in patients with schizophrenia.<sup>80,129–131</sup> The refined dopamine hypothesis postulated that excessive activation of dopamine receptors in the mesolimbic pathway (associated with positive symptoms) precipitates a reciprocal decrease in prefrontal dopaminergic signalling (cognitive symptoms). As compounds such as SKF-38393 (**25**), A-77636 (**31**) and dihydrexidine became available as tools, they were examined in multiple studies that showed that D<sub>1</sub> agonists could rescue preclinical phenotypes that were designed to model CDS in rodents or non-human primates.<sup>132</sup> Collectively, these data implied that D<sub>1</sub> agonism might have significant therapeutic potential for the treatment of impaired cognition.<sup>129</sup>

The emergence of a strong rationale for the efficacy of D<sub>1</sub> agonists in CDS came after many of the major D<sub>1</sub> agonist preclinical discovery programs had been terminated. Dihydrexidine remains available as a tool for clinical use and was examined in a CDS trial with concomitant imaging. Although the drug displayed impacts on regional cerebral blood flow in the prefrontal cortex,



no improvement in working memory performance occurred in patients.<sup>133,134</sup> The study authors have proposed that the PK properties of the molecule did not allow full exploration of the exposure range that might be necessary for efficacy, a sentiment that has been echoed by others who have highlighted the lack of oral bioavailability and short half-life of available tools as major impediments to advancing D<sub>1</sub> agonist drugs to market.<sup>135</sup> The lack of D<sub>1</sub>-selective clinical compounds has prompted researchers to use non-selective pharmacological tools to modulate dopamine signalling in exploratory CDS studies.<sup>129,80</sup> For example, bromocriptine and pergolide have been examined for their effects on the performance of schizophrenic patients on various cognitive tasks with mixed results.<sup>136</sup>

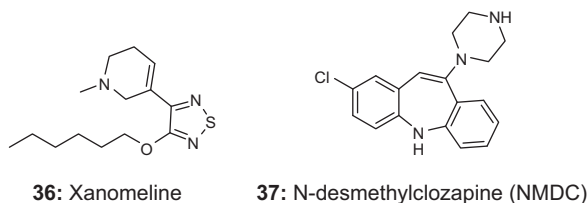
The MATRICS statement highlighting D<sub>1</sub> agonism as a promising therapeutic direction for the treatment of CDS stands as a largely untested hypothesis, and several key medicinal chemistry challenges pertaining to the development of selective D<sub>1</sub> agonists remain unsolved.<sup>3</sup> First, the presence of catecholamine functionality in all selective D<sub>1</sub> agonists reported to date imparts PK properties that have hampered broad clinical testing of the mechanism. Second, no agonist ligands are known to be selective for either D<sub>1</sub> or D<sub>5</sub>, hindering definitive disassociation of the pharmacologies of the two subtypes. Finally, the therapeutic benefits and risks of full *versus* partial agonism of D<sub>1</sub>-like receptors in the treatment of CDS remains rooted in theory and stands as another unanswered question awaiting the discovery of a suitable clinical compound.

## 6.4 Additional Mechanisms of Significant Interest in the Treatment of Cognitive Deficits in Schizophrenia

### 6.4.1 Muscarinic M<sub>1</sub> Positive Allosteric Modulators

The mAChRs have long been considered key targets for the treatment of cognitive deficits in schizophrenia that affect cholinergic neurotransmission in the forebrain.<sup>137</sup> Five mAChRs (M<sub>1</sub>–M<sub>5</sub>) exist, and knockout studies in mice suggest that the M<sub>1</sub> mAChR is the subtype affiliated with cognition and attention mechanisms.<sup>138</sup> Generating ligands that activate this subtype has been the major medicinal chemistry challenge in this arena, and it has been hampered at the orthosteric binding site by the high degree of homology within the site.<sup>139</sup> Evidence suggests that the cholinergic side-effects such as bradycardia and gastrointestinal distress that accompany the dosing of non-selective muscarinic agonists are mediated by the peripheral effects of M<sub>2</sub> and M<sub>3</sub> mAChRs.<sup>140,141</sup> The first generation of M<sub>1</sub> mAChRs activators to enter clinical trials were derived using the orthosteric agonist approach. Work to overcome the major challenge of selectivity for the five mAChR subtypes produced ligands that were thought to prefer M<sub>1</sub>, such as xanomeline (**36**), which after further evaluation were established to be mixed M<sub>1</sub>/M<sub>4</sub> agonists. Xanomeline





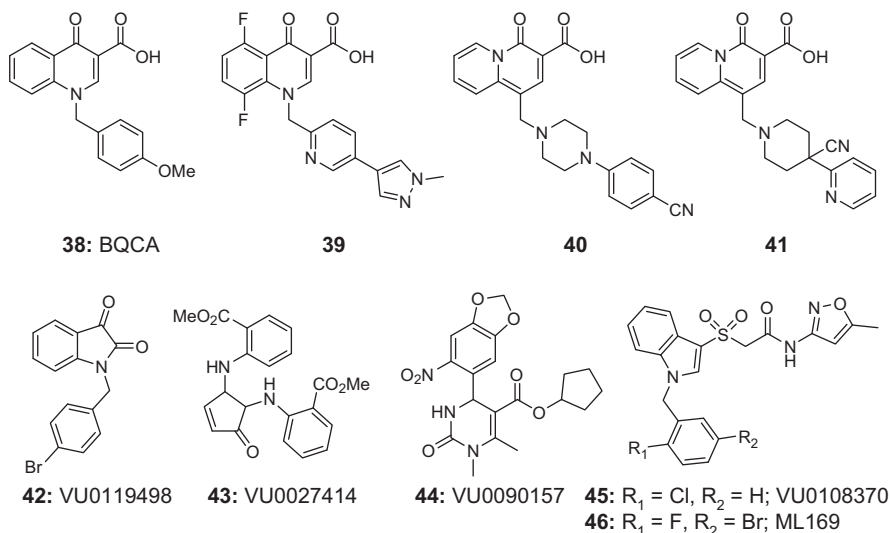
**Figure 6.10** Structures of muscarinic agonists.

(36) demonstrated compelling clinical efficacy in phase II/III clinical trials in patients with schizophrenia<sup>142</sup> but, as with many of the orthosteric agonists, it was discontinued owing to undesired cholinergic side-effects (Figure 6.10). In addition to compounds such as xanomeline (36), the *N*-desmethyl analogue of clozapine (NMDC; 37) has been identified as an allosteric agonist of the  $M_1$  mAChR and is hypothesized to contribute to the cognitive benefit of clozapine in the clinical setting.<sup>143</sup>

In an effort to overcome the medicinal chemistry challenges of identifying a selective orthosteric agonist for the  $M_1$  mAChR, researchers have turned to allosteric approaches for the identification of selective agents.<sup>144–145</sup> Two approaches have been taken to identify allosteric ligands: PAMs and allosteric agonists. Both approaches are discussed in this section with a focus on key compounds that address the primary challenge of selectivity for the mAChRs. Similar to those described above for the  $\alpha 7$  nAChRs, PAMs of mAChRs require ACh to activate the receptor.

Numerous PAMs for  $M_1$  have been identified in the literature, and the outstanding work of investigators at Vanderbilt University and Merck has advanced this concept significantly. The Merck group initially reported a series of benzyl quinolone carboxylic acids identified through the HTS lead BQCA (38; Figure 6.11).<sup>146–147</sup> This hit was established as an allosteric modulator through extensive *in vitro* evaluation, molecular modelling, and site-directed mutagenesis studies establishing its site of action.<sup>146,148</sup> A series of SAR papers document efforts to increase potency, decrease protein binding, increase CNS penetration and improve PK properties of the series. These studies produced a series of biaryl and hetero aryl quinolone carboxylic acids (39)<sup>149</sup> that were later followed by piperazine<sup>150</sup> and piperidine analogues that merged desirable features into high-quality molecules that are active *in vivo* (40).<sup>151</sup> Compound 41, for example, has been shown to be selective for  $M_1$  over  $M_2$ – $M_4$  and demonstrate robust PK characteristics in rats, dogs and primates. In addition, a potentiation assay demonstrated that the compound produced the expected leftward shift with increasing modulator concentration in an ACh dose–response curve, and *in vivo* efficacy was observed with compound 41 in a contextual fear-conditioning model at 3  $\mu$ M plasma concentration levels.

The group at Vanderbilt has produced several series of allosteric modulators of the mAChRs, including  $M_1$  (see Figure 6.11), identifying initial hits from HTS including VU0119498 (42), VU0027414 (43), VU0090157 (44) and VU0108370 (45).<sup>152,153</sup> This initial series of tools clearly expands the potential

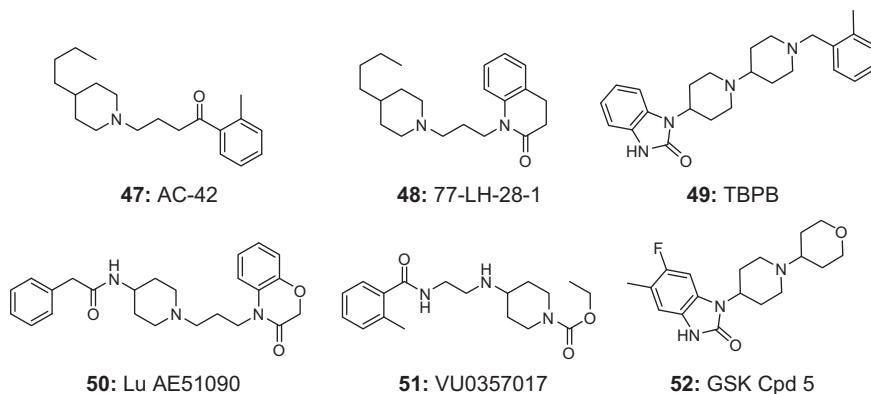


**Figure 6.11** Structures of muscarinic M<sub>1</sub> positive allosteric modulators.

for PAM approaches and establishes that structurally distinct mechanisms can have strikingly different potentials to regulate receptor coupling and downstream signalling pathways.<sup>152</sup> Work with several of these series has improved potency, but low levels of brain penetration and modest efficacy (~60% ACh maximum response) have hampered the full development of *in vivo* tools. Using library and targeted singleton syntheses, the team at Vanderbilt improved the weak and selective M<sub>1</sub> PAM VU0108370 (**45**; ~13  $\mu$ M) into the potent, selective and brain-penetrating PAM ML169 (**46**; 1.38  $\mu$ M; 84% ACh maximum).<sup>153</sup> ML169 (**46**) and compound **41** from Merck should prove to be excellent M<sub>1</sub> PAMs that enable the full profiling of this mechanism in the pursuit of cognitive approaches to schizophrenia.

### 6.4.2 Muscarinic M<sub>1</sub> Allosteric Agonists

In addition to seeking PAM approaches to the M<sub>1</sub> mAChR, investigators are searching for a selective allosteric agonist, a ligand that binds at a site that differs from that of the endogenous ligand but activates the receptor in the absence of ACh. This approach is of significant interest owing to the high degree of homology at the orthosteric site and the known difficulty in developing selective ligands (Figure 6.12). Numerous allosteric agonists initially thought to be selective for M<sub>1</sub> have been identified, including AC-42 (**47**),<sup>154</sup> 77-LH-28-1 (**48**)<sup>155</sup> and TBPB (**49**).<sup>156</sup> These ligands have enabled a deeper understanding of M<sub>1</sub> pharmacology and have established alternative binding modes including bitopic binding mechanisms.<sup>139,157</sup> A research group at Lundbeck recently identified an HTS series similar to 77-LH-28-1 (**48**) that yielded the potent and functionally selective agonist Lu AE51090 (**50**;



**Figure 6.12** Structures of muscarinic M<sub>1</sub> allosteric agonists.

EC<sub>50</sub> = 61 nM; 83% E<sub>max</sub>).<sup>158</sup> In the disclosed SAR, the critical nature of screening for M<sub>2</sub>–M<sub>5</sub> subtype selectivity is demonstrated, as it appears that this compound has been identified as a clear outlier in the series of compounds with regard to its selectivity as many close-in analogues were non-selective across mAChRs. Lu AE51090 was evaluated using the procognitive model delayed alternative Y-maze and has shown activity at free brain concentrations of about three times the *in vitro* EC<sub>50</sub>.<sup>158</sup>

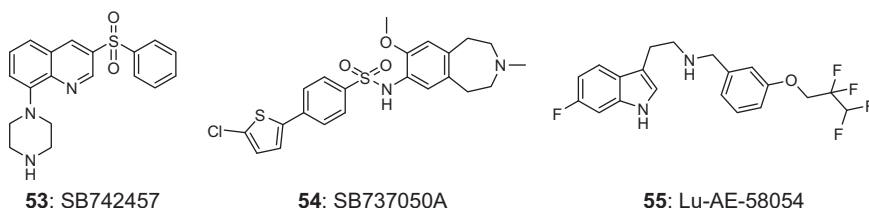
A group of investigators at GSK recently identified compound **52** *via* virtual screening of their compound file (see Figure 6.12). The core structure of **52** is similar to that of TBPB (**49**), and through a series of SAR manipulations they produced an allosteric agonist with excellent potency, selectivity, PK characteristics and CNS penetration (pEC<sub>50</sub> = 8.1 nM; intrinsic activity = 94%).<sup>159</sup> Compound **52** has been shown to enhance cell firing significantly in the hippocampus CA1 region (1 mg/kg iv) and reverse a scopolamine deficit in a schizophrenia model.<sup>159</sup> The Vanderbilt group has also identified a series of allosteric agonists, most recently characterized by VU0357017 (**51**),<sup>160</sup> a highly selective (no activity at >30 μM at other mAChRs) and potent M<sub>1</sub> agonist (EC<sub>50</sub> = 198 nM; %ACh<sub>max</sub> = 81). Extensive *in vitro* evaluation of VU0357017 (**51**), including mutagenicity studies and competition binding experiments, established that the compound was indeed an allosteric activator of M<sub>1</sub> and not an orthosteric agonist. VU0357017 (**51**) was also tested *in vivo* and demonstrated activity in a rodent model of contextual fear conditioning, reversing the deficit induced by scopolamine. With these new and selective tools in hand, the path to novel therapeutic advances for schizophrenia utilizing M<sub>1</sub> mAChR activation can be further evaluated to understand its potential.

### 6.4.3 Serotonin Subtype 6 (5-HT<sub>6</sub>) Receptor Antagonists

The modulation of serotonin levels and serotonin signalling has been an extraordinarily productive area for drug therapy. Seven serotonin subtypes

(5-HT<sub>1</sub> through 5-HT<sub>7</sub>) are known, and 5-HT<sub>6</sub> is one of the last subtypes to be cloned, expressed and studied.<sup>161</sup> Positively coupled to cyclic adenosine monophosphate (cAMP), 5-HT<sub>6</sub> is highly localized to the brain hippocampus and cortex regions, which are clearly associated with cognitive function. From a neuroanatomical beginning, various studies, including preclinical models of cognition, pointed to a utility of 5-HT<sub>6</sub> antagonists for improving cognitive function in schizophrenia, Alzheimer's disease (AD) and obesity and for other indications including epilepsy and depression; the biological underpinnings of these hypotheses have been reviewed.<sup>131,162</sup> Blockade of 5-HT<sub>6</sub> receptors increases neurotransmission *via* enhanced ACh and glutamate signalling, an observation that further supports a role for 5-HT<sub>6</sub> antagonists in restoring deficient signalling within these two systems, which is often implicated in impaired cognition.<sup>163</sup>

Although many compounds interact with the 5-HT<sub>6</sub> receptor, the SAR of potent and selective antagonists has a narrow scope. The sulfonamide moiety has been a key element in the majority of 5-HT<sub>6</sub> antagonist leads because it imparts both potency and subtype selectivity when properly incorporated.<sup>164</sup> A major SAR challenge in this area has been maintaining a polar sulfonamide functionality within molecules that have good brain penetration. The SAR relationships of the arylsulfonamide 5-HT<sub>6</sub> ligands have been reviewed, and most potent molecules maintain a somewhat conserved relationship between the arylsulfonamide group and a terminal basic amine.<sup>165</sup> A main clinical focus for the mechanism has been the symptomatic treatment of dementia associated with AD, although the potential link to CDS has also been maintained in many of the published preclinical studies (Figure 6.13). Second-generation compounds from GSK (GSK-742457 [**53**]; human 5-HT<sub>6</sub> K<sub>i</sub> < 1 nM) and SB737050A (**54**; human 5-HT<sub>6</sub> K<sub>i</sub> = 62 nM) have entered clinical trials to test their efficacy for improving impaired cognition.<sup>166,167</sup> The compound known as Lu-AE-58054 (**55**; human 5-HT<sub>6</sub> K<sub>i</sub> = 0.8 nM) is a substituted tryptamine that was examined in a phase II CDS trial in which it reportedly failed to show efficacy on the trial measures.<sup>168,169</sup> This compound is unusual in that it lacks the arylsulfonamide. Numerous medicinal chemistry publications describe selective 5-HT<sub>6</sub> antagonists with activity in rodent models of working or visual memory, with at least a dozen advancing into early clinical studies.<sup>164,170</sup> Several additional molecules with undisclosed structures have advanced into efficacy studies, and the current challenge is to validate those preclinical observations in a clinical setting.

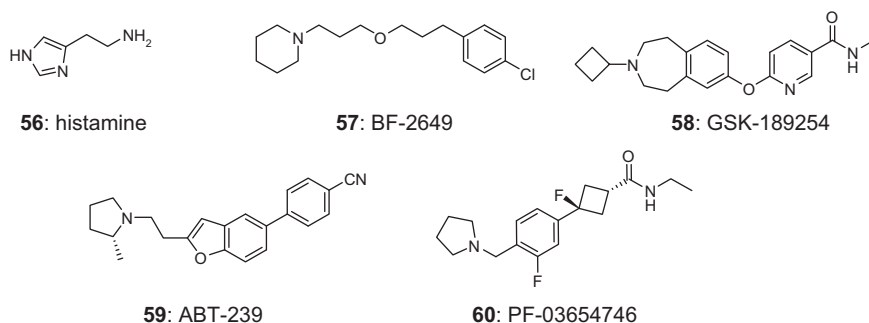


**Figure 6.13** Structures of serotonin subtype 6 receptor (5-HT<sub>6</sub>) antagonists.

### 6.4.4 Histamine H3 Receptor Antagonists

Four histamine receptor subtypes (H1–H4) have been identified. Antagonists of H1 and H2 have yielded marketed drugs for the suppression of allergy symptoms and dyspepsia, and the discovery of the H3 subtype in 1983 precipitated major biology efforts to investigate the mechanistic relevance of this target in numerous drug-discovery programs. The localization of the H3 receptor on presynaptic histaminergic neurons and its function as an auto-receptor that negatively regulates histamine release gave rise to a host of theoretical hypothesis for the use of H3 antagonism in the treatment of pain, cardiovascular disease, obesity, anxiety and cognitive impairment associated with psychiatric illness (CDS).<sup>171,172</sup> An H3 antagonist induces either blockade or inverse agonism of the negative auto-receptors, which disinhibits the release of histamine and increases histamine neurotransmission. The downstream signalling of histamine has been extensively reviewed, and numerous publications detail a preclinical association between increased histamine levels (*via* H3 antagonism) and improved performance on diverse memory and learning tasks in rodents. Similarly, H3 antagonists effectively reverse scopolamine-induced memory deficits and promote attention in rodent models.<sup>173</sup> The discovery and SAR of both early and more recent H3 antagonist leads have been reviewed.<sup>174,175</sup>

Early H3 antagonist leads were derivatives of histamine itself, and therefore contained the imidazole functionality. The activity of these molecules was hampered by PK liabilities including high clearance, low bioavailability and a strong inhibition of P450s. Most of these liabilities were connected to the imidazole structure, and the search for selective H3 antagonists that lacked this moiety became a competitive endeavour that has also been reviewed.<sup>176,177</sup> Researchers at several companies and academic consortiums have identified and optimized non-imidazole starting leads to potent H3 antagonists.<sup>178–180</sup> An academic consortium progressed through a series of SAR studies of the multiple disclosed non-imidazole H3 antagonists to enter clinical development (Figure 6.14) to arrive at BF-2649 (**57**; pitolisant; human H3  $K_i$  = 5 nM).<sup>181</sup> A GSK group optimized their SAR study to discover GSK-189254 (**58**; human

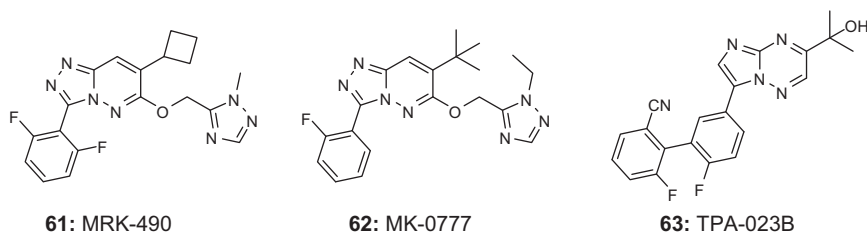


**Figure 6.14** Structures of histamine H3 receptor antagonists.

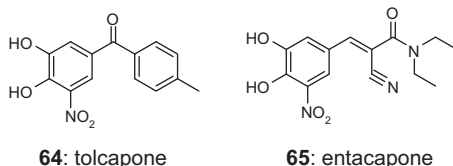
H3  $K_i = < 1 \text{ nM}$ ), which entered the clinic;<sup>182</sup> researchers in an Abbott program produced several clinical candidates including ABT-239 (**59**; rat H3  $K_i = 3 \text{ nM}$ );<sup>183</sup> and a group at Pfizer entered the clinic with PF-03654746 (**60**).<sup>184</sup> These molecules share general similarities, including an extended structure, the presence of a tertiary amine, and a non-polar terminal aromatic group. A handful of potent and selective H3 antagonists remain in human efficacy studies, many of which have undisclosed structures. To date, negative results from trials in attention deficit hyperactivity disorder (ADHD), excessive daytime sleepiness/narcolepsy and CDS with compounds such as MK-0249, PF-03654746 and GSK-189254 have focused ongoing clinical efforts toward AD-related cognitive decline as an indication. The clinical tools appear to be available to test the hypothesis that H3 antagonists may be useful for the treatment of CDS.

### 6.4.5 $\gamma$ -Aminobutyric Acid A Receptor Subtype Selective Agonists (GABA<sub>A</sub>)

GABA is the main neurotransmitter that mediates inhibitory action in the brain. A number of drugs, including anxiolytics and sedatives, interact with the GABA receptor, typically by binding to an allosteric activation site. The GABA receptor mediates inhibitory action as a complex with a chloride ion channel, and the modulatory sites act *via* allosteric binding to increase the functional activation of the complex. The GABA receptor forms a pentamer, and a number of subunit compositions occur in these complexes and interact with marketed benzodiazepine drugs. From preclinical observations, researchers began to hypothesize that particular combinations of subunits were associated with subsets of GABA effects, which might allow for the dissociation of undesirable sedation from putative cognitive benefits.<sup>185–187</sup> In particular, the  $\alpha 1$  subunit was deemed most responsible for the pronounced sedation observed with GABA PAMs both preclinically and in the clinic.<sup>188</sup> Similarly, some of the beneficial effects including anxiolytic action and normalization of impaired cognition were hypothesized to come from positive allosteric interactions at the  $\alpha 2$ ,  $\alpha 3$  or both subunits. These observations spurred interest in subunit-selective GABA PAMs (Figure 6.15).<sup>130,189</sup> Investigators at Merck, in particular, maintained a significant effort to pursue these compounds over



**Figure 6.15** Structures of  $\gamma$ -aminobutyric acid positive allosteric modulators.



**Figure 6.16** Structures of catechol-*O*-methyl transferase inhibitors.

several years. An early result from this program, MRK-490 (**61**), entered the clinic and was sedating, presumably owing to residual activity at the GABA  $\alpha 1$  subunit.<sup>188,190</sup> Refinement of the structure led to MK-0777 (**62**; GABA<sub>A</sub>  $\alpha_{2/3}$  affinity < 1 nM).<sup>191–193</sup> This compound is an antagonist at  $\alpha 1$  but retains partial PAM activity at  $\alpha 2$  and  $\alpha 3$ . Initial clinical results with MK-0777 (**62**) were promising for cognitive improvement; however, a larger study in CDS showed no benefit. Further SAR studies at Merck led to TPA-023B (**63**), with a modified heterocyclic scaffold.<sup>194</sup> This analogue has additional PAM activity at  $\alpha 2$  and  $\alpha 3$  and no functional activity at GABA  $\alpha 1$ .

#### 6.4.6 Catechol-*O*-methyl Transferase Inhibitors (COMT)

Several ongoing clinical studies are examining the effect of the catechol-*O*-methyl transferase inhibitor (COMT) tolcapone (**64**; COMT enzyme inhibition  $K_i = 8$  nM) as an add-on treatment to improve cognitive function in schizophrenic patients (Figure 6.16).<sup>195,196</sup> Tolcapone (**64**) and entacapone (**65**; COMT enzyme inhibition  $K_i = 11$  nM)<sup>197</sup> were developed as anti-Parkinsonian drugs in the 1990s, and the medicinal chemistry of COMT inhibitors has been reviewed.<sup>198</sup> Replicated findings link COMT inhibitors to small degrees of clinical improvement in working memory performance or executive function.<sup>199–201</sup> Owing to the lack of a dopamine reuptake transporter in the prefrontal cortex, COMT is the major degradation pathway for prefrontal dopamine. A functional polymorphism in the COMT gene produces a four-fold change in the activity of this metabolic enzyme and leads to prefrontal dopamine levels that vary by genotype.<sup>202</sup> Thus, the rationale for their use in CDS is related to the dopamine hypothesis of schizophrenia and to the rationale for the use of D<sub>1</sub> agonists in CDS.<sup>203</sup> Several studies estimate that COMT genotype accounts for 4–7% of the variance in performance of working memory tasks in humans.<sup>204</sup> Although the overall effect may be modest, the data suggest that COMT genotype may predict response to COMT inhibitors.<sup>202,205</sup> Specifically, it could be surmised that a COMT inhibitor such as tolcapone may amplify improvement on measures of cognitive performance in a subset of patients that have the val/val genotype.<sup>206</sup>

## References

1. T. R. E. Barnes and S. Dursun, *Psychiatry*, 2008, **7**(11), 487–490.
2. S. R. Marder and W. Fenton, *Schizophr. Res.*, 2004, **72**, 5–9.



3. R. W. Buchanan, M. Davis, D. Goff, M. F. Green, R. S. E. Keefe, A. C. Leon, K. H. Nuechterlein, T. Laughren, R. Levin, E. Stover, W. Fenton and S. R. Marder, *Schizophr. Bull.*, 2005, **31**(1), 5–19.
4. E. D. Levin and B. B. Simon, *Psychopharmacology (Berl.)*, 1998, **138**(3), 217–230.
5. S. P. Arneric and J. D. Brioni, *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities*, Wiley-Liss, New York, 1999.
6. J. A. Dani, *Biol. Psychiatry*, 2001, **49**(3), 166–174.
7. M. R. Picciotto, B. J. Caldarone, S. L. King and V. Zachariou, *Neuropsychopharmacology*, 2000, **22**, 451–465.
8. R. Freedman, H. Coon, M. Myles-Worsley, A. Orr-Urtreger, A. Olincy, A. Davis, M. Polymeropoulos, J. Holik, J. Hopkins, M. Hoff, J. Rosenthal, M. C. Waldo, F. Reimherr, P. Wender, J. Yaw, D. A. Young, C. R. Breese, C. Adams, D. Patterson, L. E. Adler, L. Kruglyak, S. Leonard and W. Byerley, *Proc. Natl. Acad. Sci. USA*, 1997, **94**(2), 587–592.
9. H. T. Nagamoto, L. E. Adler, K. A. McRae, P. Huettl, E. Cawthra, G. Gerhardt, R. Hea and J. Griffith, *Neuropsychobiology*, 1999, **39**(1), 10–17.
10. P. J. Corringer, S. Bertrand, S. Bohler, S. J. Edelstein, J. P. Changeux and D. Bertrand, *J. Neurosci.*, 1998, **18**(2), 648–657.
11. J. A. Dani and D. Bertrand, *Annu. Rev. Pharmacol. Toxicol.*, 2007, **47**, 699–729.
12. B. E. McKay, A. N. Placzek and J. A. Dani, *Biochem. Pharmacol.*, 2007, **74**(8), 1120–1133.
13. M. Alkondon, E. F. Pereira, W. S. Cortes, A. Maelicke and E. X. Albuquerque, *Eur. J. Neurosci.*, 1997, **9**(12), 2734–2742.
14. A. Mike, N. G. Castro and E. X. Albuquerque, *Brain Res.*, 2000, **882**(1–2), 155–168.
15. P. C. Astles, S. R. Baker, J. R. Boot, L. M. Broad, C. P. Dell and M. Keenan, *Curr. Drug Targets CNS Neurol. Disord.*, 2002, **1**(4), 337–348.
16. S. R. Breining, A. A. Mazurov and C. H. Miller, *Annu. Rep. Med. Chem.*, 2005, **40**, 3–16.
17. W. H. Bunnelle, M. J. Dart and M. R. Schrimpf, *Curr. Top. Med. Chem.*, 2004, **4**(3), 299–334.
18. C. Chiamulera and G. Fumagalli, *Cent. Nerv. Syst. Agents Med. Chem.*, 2007, **7**, 269–288.
19. S. L. Cincotta, M. S. Yorek, T. M. Moschak, S. R. Lewis and J. S. Rodefer, *Curr. Opin. Investig. Drugs*, 2008, **9**(1), 47–56.
20. M. Hajos and B. N. Rogers, *Curr. Pharm. Des.*, 2010, **16**, 538–554.
21. S. N. Haydar and J. Dunlop, *Curr. Top. Med. Chem.*, 2010, **10**(2), 144–152.
22. A. A. Jensen, B. Frolund, T. Liljefors and P. Krogsgaard-Larsen, *J. Med. Chem.*, 2005, **48**(15), 4705–4745.
23. A. P. Lightfoot, J. N. Kew and J. Skidmore, *Prog. Med. Chem.*, 2008, **46**, 131–171.
24. A. Mazurov, T. Hauser and C. H. Miller, *Curr. Med. Chem.*, 2006, **13**(13), 1567–1584.

25. M. N. Romanelli and F. Gualtieri, *Med. Res. Rev.*, 2003, **23**(4), 393–426.
26. J. D. Schmitt, *Curr. Med. Chem.*, 2000, **7**(8), 749–800.
27. J. Toyohara and K. Hashimoto, *Open Med. Chem. J.*, 2010, **4**, 37–56.
28. C. M. de Fiebre, E. M. Meyer, J. C. Henry, S. I. Muraskin, W. R. Kem and R. L. Papke, *Mol. Pharmacol.*, 1995, **47**(1), 164–171.
29. E. M. Meyer, E. T. Tay, R. L. Papke, C. Meyers, G. L. Huang and C. M. de Fiebre, *Brain Res.*, 1997, **768**(1–2), 49–56.
30. C. A. Briggs, D. J. Anderson, J. D. Brioni, J. J. Buccafusco, M. J. Buckley, J. E. Campbell, M. W. Decker, D. Donnelly-Roberts, R. L. Elliott, M. Gopalakrishnan, M. W. Holladay, Y. H. Hui, W. J. Jackson, D. J. Kim, K. C. Marsh, A. O'Neill, M. A. Prendergast, K. B. Ryther, J. P. Sullivan and S. P. Arneric, *Pharmacol. Biochem. Behav.*, 1997, **57**(1–2), 231–41.
31. H. Kitagawa, T. Takenouchi, R. Azuma, K. A. Wesnes, W. G. Kramer, D. E. Clody and A. L. Burnett, *Neuropsychopharmacology*, 2003, **28**(3), 542–551.
32. A. Olincy, J. G. Harris, L. L. Johnson, V. Pender, S. Kongs, D. Allensworth, J. Ellis, G. O. Zerbe, S. Leonard, K. E. Stevens, J. O. Stevens, L. Martin, L. E. Adler, F. Soti, W. R. Kem and R. Freedman, *Arch. Gen. Psychiatry*, 2006, **63**(6), 630–638.
33. R. Freedman, A. Olincy, R. W. Buchanan, J. G. Harris, J. M. Gold, L. Johnson, D. Allensworth, A. Guzman-Bonilla, B. Clement, M. P. Ball, J. Kutnick, V. Pender, L. F. Martin, K. E. Stevens, B. D. Wagner, G. O. Zerbe, F. Soti and W. R. Kem, *Am. J. Psychiatry*, 2008, **165**(8), 1040–1047.
34. K. Hashimoto, *Clin. Psychopharmacol. Neurosci.*, 2009, **7**(1), 26–27.
35. G. Mullen, J. Napier, M. Balestra, T. DeCory, G. Hale, J. Macor, R. Mack, J. Loch, 3<sup>rd</sup>, E. Wu, A. Kover, P. Verhoest, A. Sampognaro, E. Phillips, Y. Zhu, R. Murray, R. Griffith, J. Blosser, D. Gurley, A. Machulskis, J. Zongrone, A. Rosen and J. Gordon, *J. Med. Chem.*, 2000, **43**(22), 4045–4050.
36. R. Tatsumi, M. Fujio, S. Takanashi, A. Numata, J. Katayama, H. Satoh, Y. Shiigi, J. Maeda, M. Kuriyama, T. Horikawa, T. Murozono, K. Hashimoto and H. Tanaka, *J. Med. Chem.*, 2006, **49**(14), 4374–4383.
37. S. A. Castner, T. Hudzik, D. L. Maier, L. Mrzljak, T. Piser, J. S. Smith, D. Widzowski and G. V. Williams, WO2008115139A1, 2008.
38. S. Sydserff, E. J. Sutton, D. Song, M. C. Quirk, C. Maciag, C. Li, G. Jonak, D. Gurley, J. C. Gordon, E. P. Christian, J. J. Doherty, T. Hudzik, E. Johnson, L. Mrzljak, T. Piser, G. N. Smagin, Y. Wang, D. Widzowski and J. S. Smith, *Biochem. Pharmacol.*, 2009, **78**(7), 880–888.
39. S. A. Castner, G. N. Smagin, T. M. Piser, Y. Wang, J. S. Smith, E. P. Christian, L. Mrzljak and G. V. Williams, *Biol. Psychiatry*, 2011, **69**(1), 12–18.
40. J. L. Werkheiser, S. Sydserff, S. J. Hubbs, M. Ding, M. S. Eisman, D. Perry, A. J. Williams, J. S. Smith, L. Mrzljak and D. L. Maier, *Neuroscience*, 2011, **186**, 76–87.
41. D. Guendisch, M. Andrae, L. Munoz and M. C. Tilotta, *Bioorg. Med. Chem.*, 2004, **12**, 4953–4962.

42. J. Macor and E. Wu, WO9730998A1, 1997.
43. R. Tatsumi, M. Fujio, H. Satoh, J. Katayama, S. Takanashi, K. Hashimoto and H. Tanaka, *J. Med. Chem.*, 2005, **48**(7), 2678–2686.
44. R. Tatsumi, K. Seio, M. Fujio, J. Katayama, T. Horikawa, K. Hashimoto and H. Tanaka, *Bioorg. Med. Chem. Lett.*, 2004, **14**(14), 3781–3784.
45. A. L. Bodnar, L. A. Cortes-Burgos, K. K. Cook, D. M. Dinh, V. E. Groppi, M. Hajos, N. R. Higdon, W. E. Hoffmann, R. S. Hurst, J. K. Myers, B. N. Rogers, T. M. Wall, M. L. Wolfe and E. Wong, *J. Med. Chem.*, 2005, **48**(4), 905–908.
46. M. Hajos, R. S. Hurst, W. E. Hoffmann, M. Krause, T. M. Wall, N. R. Higdon and V. E. Groppi, *J. Pharmacol. Exp. Ther.*, 2005, **312**(3), 1213–1222.
47. D. P. Walker, D. G. Wishka, D. W. Piotrowski, S. Jia, S. C. Reitz, K. M. Yates, J. K. Myers, T. N. Vetman, B. J. Margolis, E. J. Jacobsen, B. A. Acker, V. E. Groppi, M. L. Wolfe, B. A. Thornburgh, P. M. Tinholt, L. A. Cortes-Burgos, R. R. Walters, M. R. Hester, E. P. Seest, L. A. Dolak, F. Han, B. A. Olson, L. Fitzgerald, B. A. Staton, T. J. Raub, M. Hajos, W. E. Hoffmann, K. S. Li, N. R. Higdon, T. M. Wall, R. S. Hurst, E. H. Wong and B. N. Rogers, *Bioorg. Med. Chem.*, 2006, **14**(24), 8219–8248.
48. D. G. Wishka, D. P. Walker, K. M. Yates, S. C. Reitz, S. Jia, J. K. Myers, K. L. Olson, E. J. Jacobsen, M. L. Wolfe, V. E. Groppi, A. J. Hanchar, B. A. Thornburgh, L. A. Cortes-Burgos, E. H. Wong, B. A. Staton, T. J. Raub, N. R. Higdon, T. M. Wall, R. S. Hurst, R. R. Walters, W. E. Hoffmann, M. Hajos, S. Franklin, G. Carey, L. H. Gold, K. K. Cook, S. B. Sands, S. X. Zhao, J. R. Soglia, A. S. Kalgutkar, S. P. Arneric and B. N. Rogers, *J. Med. Chem.*, 2006, **49**(14), 4425–4436.
49. C. L. Shaffer, M. Gunduz, R. J. Scialis and A. F. Fang, *Drug Metab. Dispos.*, 2007, **35**(7), 1188–1195.
50. B. N. Rogers, in *Agonists of alpha 7 nAChRs for the Potential Treatment of Cognitive Deficits in Schizophrenia*, Nicotinic Acetylcholine Receptors as Therapeutic Targets: Emerging Frontiers in Basic Research and Clinical Sciences, San Diego, CA, Oct. 31–Nov. 2, San Diego, CA, 2007.
51. B. N. Rogers, E. J. Jacobsen, C. J. O'Donnell, C. L. Shaffer, D. P. Walker and D. G. Wishka, *Identification of  $\alpha 7$  Nicotinic Acetylcholine Receptor Agonists for Their Assessment in Improving Cognition in Schizophrenia*, Royal Society of Chemistry, Cambridge, 2011, Vol. 4, pp. 332–362.
52. F. G. Boess, J. De Vry, C. Erb, T. Flessner, M. Hendrix, J. Luithle, C. Methfessel, B. Riedl, K. Schnizler, F. J. van der Staay, M. van Kampen, W. B. Wiese and G. Koenig, *J. Pharmacol. Exp. Ther.*, 2007, **321**(2), 716–725.
53. G. Koenig, R. Chesworth, G. Shapiro, D. Hilt, M. Palfreyman and A. Barbier, WO2010059844A1, 2010.
54. B. Biton, O. E. Bergis, F. Galli, A. Nedelec, A. W. Lochead, S. Jegham, D. Godet, C. Lanneau, R. Santamaria, F. Chesney, J. Leonardon, P. Granger, M. W. Debono, G. A. Bohme, F. Sgard, F. Besnard, D. Graham, A. Coste, A. Oblin, O. Curet, X. Vige, C. Voltz, L. Rouquier,

- J. Souilhac, V. Santucci, C. Gueudet, D. Francon, R. Steinberg, G. Griebel, F. Oury-Donat, P. George, P. Avenet and B. Scatton, *Neuropsychopharmacology*, 2007, **32**(1), 1–16.
55. P. Pichat, O. E. Bergis, J. P. Terranova, A. Urani, C. Duarte, V. Santucci, C. Gueudet, C. Voltz, R. Steinberg, J. Stemmelin, F. Oury-Donat, P. Avenet, G. Griebel and B. Scatton, *Neuropsychopharmacology*, 2007, **32**(1), 17–34.
56. C. J. O'Donnell, B. N. Rogers, B. S. Bronk, D. K. Bryce, J. W. Coe, K. K. Cook, A. J. Duplantier, E. Evrard, M. Hajos, W. E. Hoffmann, R. S. Hurst, N. Maklad, R. J. Mather, S. McLean, F. M. Nedza, B. T. O'Neill, L. Peng, W. Qian, M. M. Rottas, S. B. Sands, A. W. Schmidt, A. V. Shrikhande, D. K. Spracklin, D. F. Wong, A. Zhang and L. Zhang, *J. Med. Chem.*, 2010, **53**(3), 1222–1237.
57. B. A. Acker, E. J. Jacobsen, B. N. Rogers, D. G. Wishka, S. C. Reitz, D. W. Piotrowski, J. K. Myers, M. L. Wolfe, V. E. Groppi, B. A. Thornburgh, P. M. Tinholt, R. R. Walters, B. A. Olson, L. Fitzgerald, B. A. Staton, T. J. Raub, M. Krause, K. S. Li, W. E. Hoffmann, M. Hajos, R. S. Hurst and D. P. Walker, *Bioorg. Med. Chem. Lett.*, 2008, **18**(12), 3611–3615.
58. C. J. O'Donnell, L. Peng, B. T. O'Neill, E. P. Arnold, R. J. Mather, S. B. Sands, A. Shrikhande, L. A. Lebel, D. K. Spracklin and F. M. Nedza, *Bioorg. Med. Chem. Lett.*, 2009, **19**(16), 4747–4751.
59. T. A. Hauser, A. Kucinski, K. G. Jordan, G. J. Gatto, S. R. Wersinger, R. A. Hesse, E. K. Stachowiak, M. K. Stachowiak, R. L. Papke, P. M. Lippiello and M. Bencherif, *Biochem. Pharmacol.*, 2009, **78**(7), 803–812.
60. D. Hosford, G. Dunbar, J. A. Lieberman and A. Segreti, in *The  $\alpha 7$  neuronal nicotinic receptor (NNR) agonist TC-5619 had beneficial effects and was generally well tolerated in a phase 2 trial in Cognitive Dysfunction in Schizophrenia (CDS)*, 13th International Congress on Schizophrenia Research (ICOSR), Colorado Springs, April 2011.
61. D. Feuerbach, J. Nozulak, K. Lingenhoebl, K. McAllister and D. Hoyer, *Neurosci. Lett.*, 2007, **416**(1), 61–65.
62. D. Feuerbach, K. Lingenhoebl, H. R. Olpe, A. Vassout, C. Gentsch, F. Chaperon, J. Nozulak, A. Enz, G. Bilbe, K. McAllister and D. Hoyer, *Neuropharmacology*, 2009, **56**(1), 254–263.
63. D. K. Williams, J. Wang and R. L. Papke, *Biochem. Pharmacol.*, 2011, **82**(8), 915–930.
64. R. Zwart, G. De Filippi, L. M. Broad, G. I. McPhie, K. H. Pearson, T. Baldwinson and E. Sher, *Neuropharmacology*, 2002, **43**(3), 374–384.
65. R. S. Hurst, M. Hajos, M. Raggenbass, T. M. Wall, N. R. Higdon, J. A. Lawson, K. L. Rutherford-Root, M. B. Berkenpas, W. E. Hoffmann, D. W. Piotrowski, V. E. Groppi, G. Allaman, R. Ogier, S. Bertrand, D. Bertrand and S. P. Arneric, *J. Neurosci.*, 2005, **25**(17), 4396–4405.
66. D. W. Piotrowski, B. N. Rogers, D. M. Wilhite, B. J. Margolis, T. N. Vetman, D. Sarapa, D. G. Wishka, K. M. Yates, B. Acker, E. J. Jacobsen,

- W. W. McWhorter, Jr, V. O. Badescu, W. Xu, D. P. Walker, J. K. Myers, M. B. Berkenpas, D. M. Dinh, R. S. Hurst, T. M. Wall, N. Higdon, M. Hajos, W. E. Hoffman, K. Lee and V. E. Groppi, in *Positive Allosteric Modulators of the alpha 7 Nicotinic Acetylcholine Receptor*, 13th RSC-SCI Medicinal Chemistry Symposium, Cambridge, UK, September 14–17, 2005.
67. B. N. Rogers, D. W. Piotrowski, D. M. Wilhite, B. J. Margolis, T. N. Vetman, D. Sarapa, D. G. Wishka, K. M. Yates, B. Acker, E. J. Jacobsen, W. W. McWhorter, Jr, V. O. Badescu, W. Xu, D. P. Walker, J. K. Myers, M. B. Berkenpas, D. M. Dinh, R. S. Hurst, T. M. Wall, N. Higdon, M. Hajos, W. E. Hoffman, K. Lee and V. E. Groppi, in *Positive Allosteric Modulators of the alpha 7 Nicotinic Acetylcholine Receptor*, American Chemical Society, 2008, MEDI-248.
68. D. B. Timmermann, J. H. Gronlien, K. L. Kohlhaas, E. O. Nielsen, E. Dam, T. D. Jorgensen, P. K. Ahring, D. Peters, D. Holst, J. K. Christensen, J. Malysz, C. A. Briggs, M. Gopalakrishnan and G. M. Olsen, *J. Pharmacol. Exp. Ther.*, 2007, **323**(1), 294–307.
69. C. N. Connolly and K. A. Wafford, *Biochem. Soc. Trans.*, 2004, **32**(Pt3), 529–534.
70. H. J. Ng, E. R. Whittemore, M. B. Tran, D. J. Hogenkamp, R. S. Broide, T. B. Johnstone, L. Zheng, K. E. Stevens and K. W. Gee, *Proc. Natl. Acad. Sci. USA*, 2007, **104**(19), 8059–8064.
71. L. M. Broad, R. Zwart, K. H. Pearson, M. Lee, L. Wallace, G. I. McPhie, R. Emkey, S. P. Hollinshead, C. P. Dell, S. R. Baker and E. Sher, *J. Pharmacol. Exp. Ther.*, 2006, **318**(3), 1108–1117.
72. T. Dinklo, H. Shaban, J. W. Thuring, H. Lavreysen, K. E. Stevens, L. Zheng, C. Mackie, C. Grantham, I. Vandenberk, G. Meulders, L. Peeters, H. Verachtert, E. De Prins and A. S. J. Lesage, *J. Pharmacol. Exp. Ther.*, 2011, **336**(2), 560–574.
73. J. H. Gronlien, M. Hakerud, H. Ween, K. Thorin-Hagene, C. A. Briggs, M. Gopalakrishnan and J. Malysz, *Mol. Pharmacol.*, 2007, **72**(3), 715–724.
74. J. Changeux and S. J. Edelstein, *Curr. Opin. Neurobiol.*, 2001, **11**(3), 369–377.
75. D. Bertrand and M. Gopalakrishnan, *Biochem. Pharmacol.*, 2007, **74**(8), 1155–1163.
76. J. W. Keabian and D. B. Calne, *Nature*, 1979, **277**(5692), 93–96.
77. P. A. LeWitt, *Neurology*, 2004, **62**(4), S8–S11.
78. O. Rascol, O. Blin, C. Thalamas, S. Descombes, C. Soubrouillard, P. Azulay, N. Fabre, F. Viallet, K. Lafnitzegger, S. Wright, J. H. Carter and J. G. Nutt, *Ann. Neurol.*, 1999, **45**(6), 736–741.
79. P. J. Blanchet, J. Fang, M. Gillespie, L. Sabounjian, K. W. Locke, R. Gammans, M. M. Mouradian and T. N. Chase, *Clin. Neuropharmacol.*, 1998, **21**(6), 339–343.
80. P. S. Goldman-Rakic, *Neuropsychopharmacology*, 1999, **21**(S6), S170–S180.
81. G. V. Williams and P. S. Goldman-Rakic, *Nature*, 1995, **376**(6541), 572–575.

82. O. Guillin, A. Abi-Dargham and M. Laruelle, *Int. Rev. Neurobiol.*, 2007, **78**, 1–39.
83. S. H. Snyder, *Arch. Gen. Psychiatry*, 2002, **59**(11), 983–994.
84. A. Carlsson and M. Lindqvist, *Acta Pharmacol. Toxicol. (Copenh.)*, 1963, **20**, 140–144.
85. L. Farde, F.-A. Wiesel, C. Halldin and G. Sedvall, *Arch. Gen. Psychiatry*, 1988, **45**(1), 71–76.
86. J. M. van Rossum, *Arch. Int. Pharmacodyn. Ther.*, 1966, **160**(2), 492–494.
87. J. W. Keababian, T. Agui, J. C. Van Oene, K. Shigematsu and J. M. Saavedra, *Trends Pharmacol. Sci.*, 1986, **7**, 96–99.
88. P. Seeman, *Synapse*, 1987, **1**(2), 133–152.
89. M. Yamamoto and A. H. Schapira, *Expert Rev. Neurother.*, 2008, **8**(4), 671–677.
90. D. Robaa, C. Enzensperger, S. Eldin Abulazm, M. M. Hefnawy, H. I. El-Subbagh, T. A. Wani and J. Lehmann, *J. Med. Chem.*, 2011, **54**(20), 7422.
91. R. M. Karlsson, K. R. Hefner, D. R. Sibley and A. Holmes, *Psychopharmacology (Berl.)*, 2008, **200**(1), 117–127.
92. J. G. Cannon, B. J. Demopoulos, J. P. Long, J. R. Flynn and F. M. Sharabi, *J. Med. Chem.*, 1981, **24**(3), 238–240.
93. J. McDermid and R. J. Miller, *Annu. Rep. Med. Chem.*, 1978, **13**, 11–20.
94. D. Parkes, *N. Engl. J. Med.*, 1979, **301**(6), 873–878.
95. M. Trabucchi, M. Hofmann, O. Montefusco and P. F. Spano, *Pharmacology*, 1978, **16**(1), 150–155.
96. S. Perachon, J. C. Schwartz and P. Sokoloff, *Eur. J. Pharmacol.*, 1999, **366**(2–3), 293–300.
97. K. M. O’Boyle and J. L. Waddington, *Neuropharmacology*, 1987, **26**(12), 1807–1810.
98. K. M. O’Boyle, D. E. Gaitanopoulos, M. Brenner and J. L. Waddington, *Neuropharmacology*, 1989, **28**(4), 401–405.
99. C. H. So, G. Varghese, K. J. Curley, M. M. C. Kong, M. Alijaniam, X. Ji, T. Nguyen, B. F. O’Dowd and S. R. George, *Mol. Pharmacol.*, 2005, **68**(3), 568–578.
100. P. H. Andersen, *Eur. J. Pharmacol.*, 1988, **146**(1), 113–120.
101. J. L. Neumeyer, N. S. Kula, J. Bergman and R. J. Baldessarini, *Eur. J. Pharmacol.*, 2003, **474**(2–3), 137–140.
102. J. L. Neumeyer, M. Froimowitz, R. J. Baldessarini, A. Campbell and Y. G. Gao, *J. Recept. Res.*, 1988, **8**(1–4), 83–96.
103. W. K. Brewster, D. E. Nichols, R. M. Riggs, D. M. Mottola, T. W. Lovenberg, M. H. Lewis and R. B. Mailman, *J. Med. Chem.*, 1990, **33**(6), 1756–1764.
104. M. B. Murphy and C. Murray, and G. D. Shorten, *N. Engl. J. Med.*, 2001, **345**(21), 1548–1557.
105. K. M. O’Boyle and J. L. Waddington, *Eur. J. Pharmacol.*, 1984, **98**(3–4), 433–436.



106. J. W. Weinstock, J. W. Ladd, D. L. Brush, C. K. Pfeiffer, F. R. Kuo, G. Y. Holden, K. G. Yim, N. C. F. Hahn and A. Richard, *J. Med. Chem.*, 1980, **23**(9), 973–975.
107. J. Weinstock, D. E. Gaitanopoulos, O. D. Stringer, R. G. Franz, J. P. Hieble, L. B. Kinter, W. A. Mann, K. E. Flaim and G. Gessner, *J. Med. Chem.*, 1987, **30**(7), 1166–1176.
108. J. P. Ryman-Rasmussen, D. E. Nichols and R. B. Mailman, *Mol. Pharmacol.*, 2005, **68**(4), 1039–1048.
109. C. Piffl, H. Reither and O. Hornykiewicz, *Eur. J. Pharmacol.*, 1991, **202**(2), 273–276.
110. V. J. Watts, C. P. Lawler, A. J. Gonzales, Q.-Y. Zhou, O. Civelli, D. E. Nichols and R. B. Mailman, *Synapse*, 1995, **21**(2), 177–187.
111. M. E. Meyer and J. M. Shults, *Pharmacol. Biochem. Behav.*, 1993, **46**(2), 269–274.
112. M. E. Meyer and J. M. Shults, *Pharmacol. Biochem. Behav.*, 1993, **46**(2), 269–274.
113. K. Gnanalingham, L. Smith, P. Jenner, D. Erol, A. Hunter and C. Marsden, *Psychopharmacology (Berl.)*, 1995, **117**(3), 275–286.
114. M. M. Lewis, V. J. Watts, C. P. Lawler, D. E. Nichols and R. B. Mailman, *J. Pharmacol. Exp. Ther.*, 1998, **286**(1), 345–353.
115. P. H. Andersen and J. A. Jansen, *Eur. J. Pharmacol.*, 1990, **188**(6), 335–347.
116. J. L. Neumeyer, N. Baidur, H. B. Niznik, H. C. Guan and P. Seeman, *J. Med. Chem.*, 1991, **34**(12), 3366–3371.
117. S. T. Ross, R. G. Franz, G. Gallagher, Jr, M. Brenner, J. W. Wilson, R. M. DeMarinis, J. P. Hieble and H. M. Sarau, *J. Med. Chem.*, 1987, **30**(1), 35–40.
118. D. E. Nichols and M. M. Lewis, *Med. Chem. Res.*, 2004, **13**(1), 105–114.
119. J. Hyttel and J. Arnt, *J. Neural Transm.*, 1987, **68**(3–4), 171–189.
120. C. E. Tedford, V. B. Ruperto, V. L. Coffin, M. Cohen, M. Libonati and A. Barnett, *Drug Dev. Res.*, 1992, **26**(4), 389–403.
121. M. R. Michaelides, Y. Hong, S. DiDomenico, Jr, K. E. Asin, D. R. Britton, C. W. Lin, M. Williams and K. Shiosaki, *J. Med. Chem.*, 1995, **38**(18), 3445–3447.
122. M. P. DeNinno, R. Schoenleber, R. MacKenzie, D. R. Britton, K. E. Asin, C. Briggs, J. M. Trugman, M. Ackerman, L. Artman, L. Bednarz, R. Bhatt, P. Curzon, E. Gomez, C. H. Kang, J. Stittsworth and J. W. Keabian, *Eur. J. Pharmacol.*, 1991, **199**(2), 209–219.
123. J. W. Keabian, D. R. Britton, M. P. DeNinno, R. Perner, L. Smith, P. Jenner, R. Schoenleber and M. Williams, *Eur. J. Pharmacol.*, 1992, **229**(2–3), 203–209.
124. A. G. Gulwadi, C. D. Korpinen, R. B. Mailman, D. E. Nichols, S.-Y. Sit and M. T. Taber, *J. Pharmacol. Exp. Ther.*, 2001, **296**(2), 338–344.
125. Y. C. Martin, *Int. J. Med. Chem.*, 2011, **2011**, 8.
126. K. Shiosaki, P. Jenner, K. E. Asin, D. R. Britton, C. W. Lin, M. Michaelides, L. Smith, B. Bianchi, S. DiDomenico, L. Hodges, Y. Hong,



- L. Mahan, J. Mikusa, T. Miller, A. Nikkel, M. Stashko, D. Witte and M. Williams, *J. Pharmacol. Exp. Ther.*, 1996, **276**(1), 150–60.
127. Y. C. Zheng, K. J. Marsh, R. Bertz, T. L. El-Shourbagy and A. Adjei, *Int. J. Pharm.*, 1999, **191**(2), 131–140.
128. A. Doran, R. S. Obach, B. J. Smith, N. A. Hosea, S. Becker, E. Callegari, C. Chen, X. Chen, E. Choo, J. Cianfrogna, L. M. Cox, J. P. Gibbs, M. A. Gibbs, H. Hatch, C. E. C. A. Hop, I. N. Kasman, J. LaPerle, J. Liu, X. Liu, M. Logman, D. Maclin, F. M. Nedza, F. Nelson, E. Olson, S. Rahematpura, D. Raunig, S. Rogers, K. Schmidt, D. K. Spracklin, M. Szewc, M. Troutman, E. Tseng, M. Tu, J. W. Van Deusen, K. Venkatakrishnan, G. Walens, E. Q. Wang, D. Wong, A. S. Yasgar and C. Zhang, *Drug Metab. Dispos.*, 2005, **33**(1), 165–174.
129. A. Abi-Dargham, O. Mawlawi, I. Lombardo, R. Gil, D. Martinez, Y. Huang, D. R. Hwang, J. Keilp, L. Kochan, R. Van Heertum, J. M. Gorman and M. Laruelle, *J. Neurosci.*, 2002, **22**(9), 3708–3719.
130. D. C. Goff, M. Hill and D. Barch, *Pharmacol. Biochem. Behav.*, 2011, **99**(2), 245–253.
131. T. L. Wallace, T. M. Ballard, B. Pouzet, W. J. Riedel and J. G. Wettstein, *Pharmacol. Biochem. Behav.*, 2011, **99**(2), 130–145.
132. S. A. Castner, G. V. Williams and P. S. Goldman-Rakic, *Science*, 2000, **287**(5460), 2020–2022.
133. M. S. George, C. E. Molnar, E. L. Grenesko, B. Anderson, Q. Mu, K. Johnson, Z. Nahas, M. Knable and P. Fernandes, J. Juncos, X. Huang, D. E. Nichols and R. B. Mailman, *Schizophr. Res.*, 2007, **93**(1–3), 42–50.
134. Q. Mu, K. Johnson, P. S. Morgan, E. L. Grenesko, C. E. Molnar, B. Anderson, Z. Nahas, F. A. Kozel, S. Kose, M. Knable, P. Fernandes, D. E. Nichols, R. B. Mailman and M. S. George, *Schizophr. Res.*, 2007, **94**(1–3), 332–341.
135. R. W. Buchanan, R. Freedman, D. C. Javitt, A. Abi-Dargham and J. A. Lieberman, *Schizophr. Bull.*, 2007, **33**(5), 1120–1130.
136. M. M. McClure, P. D. Harvey, M. Goodman, J. Triebwasser, A. New, H. W. Koenigsberg, L. J. Sprung, J. D. Flory and L. J. Siever, *Neuropsychopharmacology*, 2010, **35**(6), 1356–1362.
137. N. R. Mirza, D. Peters and R. G. Sparks, *CNS Drug Rev.*, 2003, **9**(2), 159–186.
138. M. J. Marino, S. T. Rouse, A. I. Levey, L. T. Potter and P. J. Conn, *Proc. Nat. Acad. Sci. USA*, 1998, **95**(19), 11465–11470.
139. J. N. Heinrich, J. A. Butera, T. Carrick, A. Kramer, D. Kowal, T. Lock, K. L. Marquis, M. H. Pausch, M. Popiolek, S.-C. Sun, E. Tseng, A. J. Uveges and S. C. Mayer, *Eur. J. Pharmacol.*, 2009, **605**(1–3), 53–56.
140. F. P. Bymaster, P. A. Carter, M. Yamada, J. Gomez, J. Wess, S. E. Hamilton, N. M. Nathanson, D. L. McKinzie and C. C. Felder, *Eur. J. Neurosci.*, 2003, **17**(7), 1403–1410.
141. J. Wess, R. M. Eglén and D. Gautam, *Nat. Rev. Drug Discov.*, 2007, **6**(9), 721–733.

142. A. Shekhar, W. Z. Potter, J. Lightfoot, J. Lienemann, S. Dube, C. Mallinckrodt, F. P. Bymaster, D. L. McKinzie and C. C. Felder, *Am. J. Psychiatry*, 2008, **165**(8), 1033–1039.
143. T. K. Rajji, H. Uchida, Z. Ismail, W. Ng, D. C. Mamo, G. Remington, B. G. Pollock and B. H. Mulsant, *J. Clin. Psychopharmacol.*, 2010, **30**(4), 431–436. 10.1097/JCP.0b013e3181e69060.
144. N. J. M. Birdsall and S. Lazareno, *Mini Rev. Med. Chem.*, 2005, **5**(6), 523–543.
145. T. M. Bridges, E. P. LeBois, C. R. Hopkins, M. R. Wood, C. K. Jones, P. J. Conn and C. W. Lindsley, *Drug News Perspect.*, 2010, **23**, 229–240.
146. L. Ma, M. A. Seager, M. Wittmann, M. Jacobson, D. Bickel, M. Burno, K. Jones, V. K. Graufelds, G. Xu, M. Pearson, A. McCampbell, R. Gaspar, P. Shughrue, A. Danziger, C. Regan, R. Flick, D. Pascarella, S. Garson, S. Doran, C. Kreatsoulas, L. Veng, C. W. Lindsley, W. Shipe, S. Kuduk, C. Sur, G. Kinney, G. R. Seabrook and W. J. Ray, *Proc. Nat. Acad. Sci. USA*, 2009, **106**(37), 15950–15955.
147. F. V. Yang, W. D. Shipe, J. L. Bunda, M. B. Nolt, D. D. Wisnoski, Z. Zhao, J. C. Barrow, W. J. Ray, L. Ma, M. Wittmann, M. A. Seager, K. A. Koeplinger, G. D. Hartman and C. W. Lindsley, *Bioorg. Med. Chem. Lett.*, 2010, **20**(2), 531–536.
148. J. K. Shirey, A. E. Brady, P. J. Jones, A. A. Davis, T. M. Bridges, J. P. Kennedy, S. B. Jadhav, U. N. Menon, Z. Xiang, M. L. Watson, E. P. Christian, J. J. Doherty, M. C. Quirk, D. H. Snyder, J. J. Lah, A. I. Levey, M. M. Nicolle, C. W. Lindsley and P. J. Conn, *J. Neurosci.*, 2009, **29**(45), 14271–14286.
149. S. D. Kuduk, C. N. Di Marco, V. Cofre, D. R. Pitts, W. J. Ray, L. Ma, M. Wittmann, M. Seager, K. Koeplinger, C. D. Thompson, G. D. Hartman and M. T. Bilodeau, *Bioorg. Med. Chem. Lett.*, 2010, **20**(2), 657–661.
150. S. D. Kuduk, R. K. Chang, C. N. Di Marco, W. J. Ray, L. Ma, M. Wittmann, M. A. Seager, K. A. Koeplinger, C. D. Thompson, G. D. Hartman and M. T. Bilodeau, *ACS Med. Chem. Lett.*, 2010, **1**(6), 263–267.
151. S. D. Kuduk, R. K. Chang, C. N. Di Marco, D. R. Pitts, T. J. Greshock, L. Ma, M. Wittmann, M. A. Seager, K. A. Koeplinger, C. D. Thompson, G. D. Hartman, M. T. Bilodeau and W. J. Ray, *J. Med. Chem.*, 2011, **54**(13), 4773–4780.
152. J. E. Marlo, C. M. Niswender, E. L. Days, T. M. Bridges, Y. Xiang, A. L. Rodriguez, J. K. Shirey, A. E. Brady, T. Nalywajko, Q. Luo, C. A. Austin, M. B. Williams, K. Kim, R. Williams, D. Orton, H. A. Brown, C. W. Lindsley, C. D. Weaver and P. J. Conn, *Mol. Pharmacol.*, 2009, **75**(3), 577–588.
153. P. R. Reid, T. M. Bridges, D. J. Sheffler, H. P. Cho, L. M. Lewis, E. Days, J. S. Daniels, C. K. Jones, C. M. Niswender, C. D. Weaver, P. J. Conn, C. W. Lindsley and M. R. Wood, *Bioorg. Med. Chem. Lett.*, 2011, **21**(9), 2697–2701.
154. C. Langmead, V. Fry, I. Forbes, C. Branch, A. Christopoulos, M. Wood and H. Herdon, *Mol. Pharmacol.*, 2006, **69**(1), 236–246.
155. C. J. Langmead, N. E. Austin, C. L. Branch, J. T. Brown, K. A. Buchanan, C. H. Davies, I. T. Forbes, V. A. H. Fry, J. J. Hagan,

- H. J. Herdon, G. A. Jones, R. Jeggo, J. N. C. Kew, A. Mazzali, R. Melarange, N. Patel, J. Pardoe, A. D. Randall, C. Roberts, A. Roopun, K. R. Starr, A. Teriakidis, M. D. Wood, M. Whittington, Z. Wu and J. Watson, *Br. J. Pharmacol.*, 2008, **154**(5), 1104–1115.
156. T. Spalding, C. Trotter, N. Skjaerbaek and T. Messier, E. Currier, E. Burstein, D. Li, U. Hacksell and M. Brann, *Mol. Pharmacol.*, 2002, **61**(6), 1297–1302.
157. V. A. Avlani, C. J. Langmead, E. Guida, M. D. Wood, B. G. Tehan, H. J. Herdon, J. M. Watson, P. M. Sexton and A. Christopoulos, *Mol. Pharmacol.*, 2010, **78**(1), 94–104.
158. A. G. Sams, M. Hentzer, G. K. Mikkelsen, K. Larsen, C. Bundgaard, N. Plath, C. T. Christoffersen and B. Bang-Andersen, *J. Med. Chem.*, 2010, **53**(17), 6386–6397.
159. B. Budzik, V. Garzya, D. Shi, G. Walker, M. Woolley-Roberts, J. Pardoe, A. Lucas, B. Tehan, R. A. Rivero, C. J. Langmead, J. Watson, Z. Wu, I. T. Forbes and J. Jin, *ACS Med. Chem. Lett.*, 2010, **1**(6), 244–248.
160. E. P. Lebois, T. M. Bridges, L. M. Lewis, E. S. Dawson, A. S. Kane, Z. Xiang, S. B. Jadhav, H. Yin, J. P. Kennedy, J. Meiler, C. M. Niswender, C. K. Jones, P. J. Conn, C. D. Weaver and C. W. Lindsley, *ACS Chem. Neurosci.*, 2009, **1**(2), 104–121.
161. F. Borsini, *Int. Rev. Neurobiol.*, 2010, **94**, xi–xii.
162. E. S. Mitchell and J. F. Neumaier, *Pharmacol. Ther.*, 2005, **108**(3), 320–333.
163. N. Upton, T. T. Chuang, A. J. Hunter and D. J. Virley, *Neurotherapeutics*, 2008, **5**(3), 458–469.
164. R. A. Glennon, U. Siripurapu, B. L. Roth, R. Kolanos, M. L. Bondarev, D. Sikazwe, M. Lee and M. Dukat, *Curr. Top. Med. Chem.*, 2010, **10**(5), 579–595.
165. D. J. Heal, S. L. Smith, A. Fisas, X. Codony and H. Buschmann, *Pharmacol. Ther.*, 2008, **117**(2), 207–231.
166. D. S. Dupuis, C. Mannoury la Cour, C. Chaput, L. Verrielle, G. Lavielle and M. J. Millan, *Eur. J. Pharmacol.*, 2008, **588**(2–3), 170–177.
167. N. Upton, T. T. Chuang, A. J. Hunter and D. J. Virley, *Neurotherapeutics*, 2008, **5**(3), 458–469.
168. J. Arnt, B. Bang-Andersen, B. Grayson, F. P. Bymaster, M. P. Cohen, N. W. DeLapp, B. Giethlen, M. Kreilgaard, D. L. McKinzie, J. C. Neill, D. L. Nelson, S. M. Nielsen, M. N. Poulsen, J. M. Schaus and L. M. Witten, *Int. J. Neuropsychopharmacol.*, 2010, **13**(8), 1021–1033.
169. S. H. Zhao, J. Berger, R. D. Clark, S. G. Sethofer, N. E. Krauss, J. M. Brothers, R. S. Martin, D. L. Misner, D. Schwab and L. Alexandrova, *Bioorg. Med. Chem. Lett.*, 2007, **17**(12), 3504–3507.
170. K. G. Liu and A. J. Robichaud, in *International Review of Neurobiology*, ed. B. Franco, Academic Press, 2010, Vol. 94, pp. 1–34.
171. G. Bongers, R. A. Bakker and R. Leurs, *Biochem. Pharmacol.*, 2007, **73**(8), 1195–1204.
172. M. J. Gemkow, A. J. Davenport, S. Harich, B. A. Ellenbroek, A. Cesura and D. Hallett, *Drug Discov. Today*, 2009, **14**(9–10), 509–515.

173. S. Miyazaki, M. Imaizumi and K. Onodera, *Life Sci.*, 1995, **57**(23), 2137–2144.
174. J. D. Brioni, T. A. Esbenshade, T. R. Garrison, S. R. Bitner and M. D. Cowart, *J. Pharmacol. Exp. Ther.*, 2011, **336**(1), 38–46.
175. E. P. Lebois, C. K. Jones and C. W. Lindsley, *Curr. Top. Med. Chem.*, 2011, **11**(6), 648–660.
176. M. Cowart, R. Altenbach, L. Black, R. Faghhi, C. Zhao and A. A. Hancock, *Mini Rev. Med. Chem.*, 2004, **4**(9), 979–992.
177. M. Cowart, J. K. Pratt, A. O. Stewart, Y. L. Bennani, T. A. Esbenshade and A. A. Hancock, *Bioorg. Med. Chem. Lett.*, 2004, **14**(3), 689–693.
178. R. L. Hudkins, R. Raddatz, M. Tao, J. R. Mathiasen, L. D. Aimone, N. C. Becknell, C. P. Prouty, L. J. Knutsen, M. Yazdanian, G. Moachon, M. A. Ator, J. P. Mallamo, M. J. Marino, E. R. Bacon and M. Williams, *J. Med. Chem.*, 2011, **54**(13), 4781–4792.
179. M. Ishikawa and K. Hashimoto, *Curr. Pharm. Des.*, 2011, **17**, 121–129.
180. G. Meier, X. Ligneau, H. H. Pertz, C. R. Ganellin, J. C. Schwartz, W. Schunack and H. Stark, *Bioorg. Med. Chem.*, 2002, **10**(8), 2535–2542.
181. X. Ligneau, D. Perrin, L. Landais, J. C. Camelin, T. P. Calmels, I. Berrebi-Bertrand, J. M. Lecomte, R. Parmentier, C. Anacleto, J. S. Lin, V. Bertaina-Anglade, C. D. la Rochelle, F. d’Aniello, A. Rouleau, F. Gbahou, J. M. Arrang, C. R. Ganellin, H. Stark, W. Schunack and J. C. Schwartz, *J. Pharmacol. Exp. Ther.*, 2007, **320**(1), 365–375.
182. A. D. Medhurst, A. R. Atkins, I. J. Beresford, K. Brackenborough, M. A. Briggs, A. R. Calver, J. Cilia, J. E. Cluderay, B. Crook, J. B. Davis, R. K. Davis, R. P. Davis, L. A. Dawson, A. G. Foley, J. Gartlon, M. I. Gonzalez, T. Heslop, W. D. Hirst, C. Jennings, D. N. Jones, L. P. Lacroix, A. Martyn, S. Ociecka, A. Ray, C. M. Regan, J. C. Roberts, J. Schogger, E. Southam, T. O. Stean, B. K. Trail, N. Upton, G. Wadsworth, J. A. Wald, T. White, J. Witherington, M. L. Woolley, A. Worby and D. M. Wilson, *J. Pharmacol. Exp. Ther.*, 2007, **321**(3), 1032–1045.
183. T. R. Miller, I. Milicic, J. Bauch, J. Du, B. Surber, K. E. Browman, K. Marsh, M. Cowart, J. D. Brioni and T. A. Esbenshade, *Br. J. Pharmacol.*, 2009, **157**(1), 139–149.
184. T. T. Wager, B. A. Pettersen, A. W. Schmidt, D. K. Spracklin, S. Mente, T. W. Butler, H. Howard, D. J. Lettiere, D. M. Rubitski, D. F. Wong, F. M. Nedza, F. R. Nelson, H. Rollema, J. W. Raggon, J. Aubrecht, J. K. Freeman, J. M. Marcek, J. Cianfrogna, K. W. Cook, L. C. James, L. A. Chatman and P. A. Iredale, M. J. Banker, M. L. Homiski, J. B. Munzner and R. Y. Chandrasekaran, *J. Med. Chem.*, 2011, **54**(21), 7602–7620.
185. D. A. Lewis, T. Hashimoto and D. W. Volk, *Nat. Rev. Neurosci.*, 2005, **6**(4), 312–324.
186. J. R. Atack, *Curr. Top. Behav. Neurosci.*, 2010, **2**, 331–360.
187. J. R. Atack, *Curr. Top. Med. Chem.*, 2011, **11**(9), 1176–1202.
188. J. R. Atack, K. A. Wafford, L. J. Street, G. R. Dawson, S. Tye, K. Van Laere, G. Bormans, S. M. Sanabria-Bohorquez, I. De Lepeleire, J. N. de

- Hoon, A. Van Hecken, H. D. Burns, R. M. McKernan, M. G. Murphy and R. J. Hargreaves, *J. Psychopharmacol.*, 2011, **25**(3), 314–328.
189. J. A. Gray and B. L. Roth, *Schizophr. Bull.*, 2007, **33**(5), 1100–1119.
190. M. Reite, *Am. J. Psychiatry*, 2011, **168**(7), 755–755.
191. J. R. Atack, K. A. Wafford, S. J. Tye, S. M. Cook, B. Sohal, A. Pike, C. Sur, D. Melillo, L. Bristow, F. Bromidge, I. Ragan, J. Kerby, L. Street, R. Carling, J. L. Castro, P. Whiting, G. R. Dawson and R. M. McKernan, *J. Pharmacol. Exp. Ther.*, 2006, **316**(1), 410–422.
192. R. W. Carling, A. Madin, A. Guiblin, M. G. Russell, K. W. Moore, A. Mitchinson, B. Sohal, A. Pike, S. M. Cook, I. C. Ragan, R. M. McKernan, K. Quirk, P. Ferris, G. Marshall, S. A. Thompson, K. A. Wafford, G. R. Dawson, J. R. Atack, T. Harrison, J. L. Castro and L. J. Street, *J. Med. Chem.*, 2005, **48**(23), 7089–7092.
193. M. G. Russell, R. W. Carling, L. J. Street, D. J. Hallett, S. Goodacre, E. Mezzogori, M. Reader, S. M. Cook, F. A. Bromidge, R. Newman, A. J. Smith, K. A. Wafford, G. R. Marshall, D. S. Reynolds, R. Dias, P. Ferris, J. Stanley, R. Lincoln, S. J. Tye, W. F. Sheppard, B. Sohal, A. Pike, M. Dominguez, J. R. Atack and J. L. Castro, *J. Med. Chem.*, 2006, **49**(4), 1235–1238.
194. J. R. Atack, D. J. Hallett, S. Tye, K. A. Wafford, C. Ryan, S. M. Sanabria-Bohorquez, W. S. Eng, R. E. Gibson, H. D. Burns, G. R. Dawson, R. W. Carling, L. J. Street, A. Pike, I. De Lepeleire, K. Van Laere, G. Bormans, J. N. de Hoon, A. Van Hecken, R. M. McKernan, M. G. Murphy and R. J. Hargreaves, *J. Psychopharmacol.*, 2011, **25**(3), 329–344.
195. N. Borges, M. A. Vieira-Coelho, A. Parada and P. Soares-Da-Silva, *J. Pharmacol. Exp. Ther.*, 1997, **282**(2), 812–817.
196. J. A. Apud, V. Mattay, J. Chen, B. S. Kolachana, J. H. Callicott, R. Rasetti, G. Alce, J. E. Iudicello, N. Akbar, M. F. Egan, T. E. Goldberg and D. R. Weinberger, *Neuropsychopharmacology*, 2007, **32**(5), 1011–1020.
197. M. Forsberg, M. Lehtonen, M. Heikkinen, J. Savolainen, T. Jarvinen and P. T. Mannisto, *J. Pharmacol. Exp. Ther.*, 2003, **304**(2), 498–506.
198. D. A. Learmonth, L. E. Kiss and P. Soares-da-Silva, *Int. Rev. Neurobiol.*, 2010, **95**, 119–162.
199. E. Nissinen and P. T. Mannisto, *Int. Rev. Neurobiol.*, 2010, **95**, 73–118.
200. A. Gordin, S. Kaakkola and H. Teravainen, *J. Neural Transm.*, 2004, **111**(10–11), 1343–1363.
201. M. J. Bonifacio, P. N. Palma, L. Almeida and P. Soares-da-Silva, *CNS Drug Rev.*, 2007, **13**(3), 352–379.
202. A. Heinz and M. N. Smolka, *Rev. Neurosci.*, 2006, **17**(3), 359–367.
203. P. Roussos, S. G. Giakoumaki and P. Bitsios, *Biol. Psychiatry*, 2009, **66**(11), 997–1004.
204. J. A. Apud and D. R. Weinberger, *CNS Drugs*, 2007, **21**(7), 535–557.
205. H. M. Lachman, *Neuropsychopharmacology*, 2008, **33**(13), 3027–3029.
206. C. W. Olanow and P. B. Watkins, *Clin. Neuropharmacol.*, 2007, **30**(5), 287–294.

## **Section 2**

# **Depression and Anxiety**





## CHAPTER 7

# *The Neurobiology of Depression and Anxiety: How Do We Change from Models of Drug Efficacy to Understanding Mood and Anxiety Disorders?*

GEORGIA E. HODES AND SCOTT J. RUSSO\*

Fishberg Department of Neuroscience and Freidman Brain Institute, Mount Sinai School of Medicine, 1425 Madison Ave., New York, NY 10029, USA

\*Email: [scott.russo@mssm.edu](mailto:scott.russo@mssm.edu)

## 7.1 Introduction

Depression and anxiety disorders affect 20.8% of people in the United States within their lifetime and can affect up to 9.5% of the population in any given year.<sup>1</sup> Women are twice as likely as men to experience mood and anxiety disorders<sup>2</sup> and have a lifetime occurrence of up to 30%.<sup>3</sup> Currently 40–60% of patients experiencing these disorders do not respond to traditional treatments; thus, there is a need for new treatments targeting the underlying biology of vulnerability to depression and anxiety.<sup>4</sup> One factor that may contribute to this inefficacy is the reliance on male animals in drug discovery work, where last year 65% of studies published in pharmacology and 55% of published studies in neuroscience used male animals exclusively.<sup>5</sup> Even when females were tested, only approximately 20% of the studies in neuroscience or pharmacology

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

actually used sex as a factor in the analysis of their data. Additionally, we have relied too heavily on the monoamine hypothesis of depression. All current available treatments act by altering levels of monoamines or catecholamines, and little has been done in the way of developing treatments to target the underlying biology of depression or anxiety over the past 50 years.<sup>6</sup>

The following chapter will provide an overview of the neurobiology of depression and anxiety disorders within the context of drug development. We begin with a historical overview of the current standard treatments for these disorders with a focus on the monoamine hypothesis of depression. Although we will focus more intensely on depression, most depressive and anxiety disorders are treated with similar antidepressant drugs and evidence points to overlapping mechanisms and brain circuits affected in both disorders. Thus, we will highlight these similarities whenever possible. We will also include a discussion about the basic research that is being conducted to develop new treatments using relevant animal models and behavioural testing by considering their strengths and weaknesses. We will then discuss the major neuroanatomy associated with depression and anxiety disorders. Bringing together the information generated from animal models and neuroanatomy studies, we will discuss new treatments that are being developed based on an understanding of some of the mechanisms involved in the etiology of these disorders. We will then conclude with a discussion of the future need for personalized treatment targeting mechanisms on an individual basis.

## **7.2 A Brief History of the Development of Current Antidepressant Treatments**

Monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants were both serendipitous discoveries.<sup>7</sup> MAOIs were developed in an attempt to increase the efficacy of the antituberculosis drug Isoniazid, but were found instead to have antidepressant properties. The tricyclic antidepressant imipramine was first synthesized as a derivative of the antipsychotic drug chlorpromazine and later found to have antidepressant properties. Based on the therapeutic effects of these drugs, a monoamine hypothesis of depression was developed.<sup>8</sup> The idea behind this theory is that depression is caused by alterations in levels of monoamines and catecholamines in the brain. However, despite years of research and approximately 30 currently available antidepressants designed to target monoamines, there is limited empirical evidence to support this. Depletion of monoamines or catecholamines in healthy humans does not induce a depressive like state; however, this may be sex specific,<sup>9</sup> since depletion of the serotonin (5-HT) precursor tryptophan induces a greater number of negative feelings and decreases mood in women but not in men. Although depletion of 5-HT does not induce depression in healthy men or women, removing tryptophan in women currently under treatment for depression results in a greater degree of depressive behaviour than in males.<sup>10</sup> Together these studies suggest that regardless of circulating levels of 5-HT the

female system has an increased susceptibility to depression. This is further supported by the report of sex differences in response to antidepressants.<sup>11</sup> Younger women (but not older women, >44 years) had a better response to fluoxetine treatment than to a tricyclic antidepressant, while men and post-menopausal women did not differ in their response to treatment. Therefore, factors such as sex and reproductive stage influence antidepressant efficacy and should be used to guide future treatment studies.

The type of antidepressant used for treatment also appears to influence the propensity for symptom relapse, further supporting the idea that dysregulation of serotonin is not necessarily the cause of depression.<sup>12</sup> The majority of subjects treated with selective serotonin reuptake inhibitors (SSRIs) or MAOIs relapsed after tryptophan depletion (90%), whereas those treated with norepinephrine-targeted antidepressants had a much lower incidence of relapse (20–25%). Depletion of catecholamines using alpha-methyl-para-tyrosine (AMPT) resulted in opposite effects; patients who were treated with desipramine had a greater incidence of relapse (80%) than those treated with fluoxetine (20–25%). Therefore, rather than causing depression, serotonin and norepinephrine (NE) may have a greater involvement with the efficacy of antidepressant treatment. That said, we will review the evidence for the role of monoamines and catecholamines in depression and anxiety because much of what is currently known about these disorders derives from this research.

### **7.2.1 Monoamines and Selective Serotonin Reuptake Inhibitors**

Antidepressants act to increase 5-HT transmission in the brain; although many of the general mechanisms are known, the exact mechanisms by which they alleviate depression are still not well understood. SSRIs act at the synapse to block reuptake of 5-HT by the pre-synaptic cell so it remains available to bind to and activate the post-synaptic cell. SSRIs work immediately on the brain to increase levels of extra-cellular 5-HT in areas such as the prefrontal cortex, dorsal striatum and hippocampus.<sup>13</sup> Since antidepressant treatment requires several weeks to become effective, it is clear that simply increasing 5-HT levels alone is not sufficient to alleviate symptoms of depression. Some of the time lag may be explained by alterations occurring at the receptor level. For example, the initial increases in 5-HT from antidepressant treatment results in negative feedback through pre-synaptic auto-receptors, which reduces the amount of 5-HT being produced.<sup>13</sup> Therefore, blocking these auto-receptors potentiates the release of 5-HT in these areas and may be a way of reducing the lag time of treatment efficacy. Conversely, post-synaptic 5-HT<sub>1A</sub> receptors are found in limbic areas such as the hippocampus, septum and cortex, and they can have both inhibitory and excitatory effects depending upon location. Chronic treatment with fluoxetine may act to desensitize 5-HT<sub>1A</sub> auto-receptors,<sup>14</sup> whereas it does not desensitize post-synaptic receptors,<sup>15</sup> indicating that antidepressants act through multiple mechanisms within this system. Finally, 5-HT itself, which is produced in cell bodies located in the median and dorsal raphe nucleus, enervates many brain structures, and acts upon more than 14 5-HT

receptor subtypes.<sup>16</sup> Therefore, an in-depth understanding of these receptors in depression and anxiety disorders may lead to treatments with greater efficacy and fewer side-effects.

In addition to the direct effects of antidepressants on pre- and post-synaptic 5-HT receptors in discrete brain regions, there is controversial evidence that they may also directly impact receptor affinity. In unmedicated human depressives, PET scans indicated lower levels of 5-HT<sub>1A</sub> receptor binding in the hippocampus.<sup>17</sup> However, preclinical studies on the acute and chronic effects of two SSRIs found that fluoxetine and citalopram differently affected 5-HT<sub>1A</sub> receptor density but not binding in adult male rats. Acute treatment with fluoxetine, but not citalopram, decreased the density of receptors.<sup>18</sup> Conversely, chronic treatment with citalopram, but not fluoxetine, increased the density of 5-HT<sub>1A</sub> receptors. While receptor density changed with antidepressant treatment there were no effects on binding affinity, suggesting that antidepressant treatments did not alter the efficacy of the receptors. In another report, chronic treatment with fluoxetine decreased 5-HT<sub>1A</sub> receptor density; however, binding affinity was unaffected.<sup>19</sup> Therefore the relationship between receptor density receptor binding affinity and antidepressant treatment remains to be determined.

### 7.2.2 Catecholamines and Tricyclic Antidepressants

Imipramine was one of the first synthesized antidepressant treatments and forms the basis for all of the tricyclic antidepressants. Tricyclic antidepressants act by blocking reuptake of NE and 5-HT into the pre-synaptic cell. The catecholamine hypothesis of affective disorders predates the monoamine hypothesis and developed from studies of isoniazid. Treatment with MAOIs increased circulating levels of NE and, to a lesser extent, serotonin. This information was paired with the fact that amphetamine, which also increased NE and dopamine, had been used as a treatment for depression. Furthermore, withdrawal from amphetamine, which depletes NE, results in a depressive-like state. Together these studies were taken as evidence that alterations in circulating levels of catecholamines resulted in depression.<sup>8</sup> Interestingly, the ability of imipramine to alleviate the symptoms of depression without regulating monoamine oxidase was originally interpreted as evidence against the catecholamine hypothesis of affective disorders. At the time the drug was developed its main mechanism of action involving the inhibition of the reuptake of the catecholamine NE was not understood.

Despite flaws, there is some validity to the catecholamine hypothesis of affective disorders. Stressful experience has been shown to activate the noradrenergic system and, in turn, it interacts with the 5-HT system.<sup>20</sup> Stressful experience triggers the firing of noradrenergic neurons in the locus coeruleus (LC), which is in an area associated with the stress response. Activation of these neurons results in increased release of NE that subsequently alters the activation of a variety of neurons.<sup>21</sup> SSRI class antidepressants may act upon the NE system by increasing serotonin levels, leading to an activation of 5-HT<sub>1A</sub>

receptors. This subsequently decreases the firing rates of NE neurons and reduces NE levels, resulting in a decrease of the LC's response to stress. Like 5-HT, NE acts on a number of receptor subtypes and has different effects depending on both the receptor subtype and receptor location.<sup>20</sup> NE  $\alpha 1$  and  $\beta$  receptors located post-synaptically on non-NE neurons can modulate the release of other neurotransmitters. NE  $\alpha 2$  receptors act like auto-receptors and when located pre-synaptically on NE neurons inhibit NE release. Post-synaptic  $\alpha 2$  receptors located on NE neurons are affected by tricyclic antidepressants and contribute to the elevations in brain NE. These same receptors can form heteroreceptors on the nerve terminals of other neurotransmitters and modulate their activity.

One of the major issues with tricyclics is the increased negative side-effect profile compared to SSRIs. Tricyclics are also potentially more dangerous because they act on receptors that are located in the heart and overdose can be fatal,<sup>22</sup> which is of particular concern given the possibility of suicidal behaviour in depressive patients. A new generation of double and triple reuptake inhibitors can boost the positive effects of altering NE and 5-HT and, in some cases, dopamine in the brain in the absence of the more dangerous peripheral side-effects. While these drugs are safer than the tricyclic antidepressants and offer an alternative to people who do not find SSRIs effective, there is little evidence that these drugs have higher rates of remission than other treatment strategies.<sup>6</sup>

## **7.3 Animal Models of Depression and Anxiety**

Most animal models of depression use stress exposure to induce behaviours thought to be relevant to depression and anxiety. These models are generally capable of reproducing one or more symptoms of mood or anxiety disorders. Relevant models must fulfil proper face, construct and predictive validity.<sup>23</sup> Face validity is the ability of a model to mimic the symptoms of the disorder. Construct validity evaluates the plausibility or rationale of the model to explain the human disorder. The predictive validity is the ability of antidepressant treatments to reverse the symptoms induced by the model. While most drug development efforts have largely ignored models that fulfil all of these multiple validities, future efforts to understand the etiology of depression and anxiety must establish these criteria for new discovery.

### **7.3.1 Chronic Stressors**

The earliest behavioural models of depression and anxiety disorders focused on maternal separation stress; some of the first included Harlow's famous studies of the effects of separating mother and infant rhesus monkey dyads.<sup>24</sup> Many of these separation experiments only studied and reported depression-associated behaviour during the period of separation. More recent studies in rodents have shown effects of maternal separation on depression- and anxiety-associated hormonal and molecular changes of the offspring once they reach adulthood.<sup>25</sup> In non-human primates, periods of maternal separation can increase adult

anxiety- and depression-associated behaviour and alter neurochemistry in subjects with a genetic variation in the serotonin transporter promoter region.<sup>26</sup> Though these studies have good face and construct validity, a major limitation for wide-scale implementation at the basic science level is that they are time consuming and expensive. Additionally, studies in rodents have demonstrated that early life stress affects male, but not female, rodents in adulthood.<sup>27</sup> Therefore, they ignore the need for models that match the greater occurrence of these disorders in the human female population.

Another well-established model of depression still being used today is learned helplessness (LH). In the earliest study,<sup>28</sup> dogs were exposed to inescapable shock and then given the opportunity to escape the shock by jumping over a barrier in a shuttle box. Without exposure to the shock, dogs quickly learned to escape, whereas animals previously exposed to the inescapable shock fail to learn and essentially “give up”. Instead of jumping over the barrier, these animals would sit or lie down and whimper quietly.<sup>29</sup> Even when the animals that had been previously exposed to shock were able to jump over the barrier, they were unable to make the association between escape and termination of the shock, so they failed to learn to escape. Additional adaptations have increased the utility of the model by including yoked controls. Here, both groups of animals tested are exposed to the shock before avoidance training but only one group can control the termination of the shock.<sup>30</sup> Animals who could actively terminate the stressor were able to learn to escape in a subsequent active avoidance task, whereas yoked animals receiving the same shock without control over termination did not learn the active avoidance task. These effects were replicated in rodent models that similarly showed there are individual differences in response.<sup>31</sup> A third of the animals exposed to inescapable shock displayed susceptibility and engaged in helplessness behaviour,<sup>32</sup> which highlights a degree of face validity to the model. The inescapable stress used in LH depletes NE and impairs active avoidance responding. However, animals exposed to controllable stress of the same intensity and duration exhibit less NE depletion and increased active avoidance in the LH paradigm.<sup>33,34</sup> Interestingly, all classes of antidepressant increase active avoidance in adult male rats,<sup>35</sup> and SSRIs even reverse the stress-induced changes in neural plasticity induced by LH.<sup>36,37</sup> However, females do not have the same stress-related behavioural deficits in LH,<sup>38–40</sup> nor do they exhibit any chronic stress-related decrease in neural plasticity.<sup>40</sup> Therefore, stress-induced alterations in the NE system may not have the same debilitating behavioural and physical effects in females.

Although the LH paradigm exhibits multiple validities and relevance to depression and antidepressant responses, it is not thought to model the underlying biological changes driving depression. Thus, it is unclear how useful LH is to screen novel antidepressant compounds. From a technical perspective, it also requires large cohorts of animals and, as mentioned previously, female rats fail to show helplessness behaviour when tested with an active avoidance task.<sup>41,42</sup> That said, LH exhibits a high degree of face and predictive validity and is very useful for studies utilizing existing antidepressants.<sup>43</sup>



A recent popular method of inducing a depression-like phenotype in animals is chronic mild stress (CMS). In this paradigm, animals are exposed to variable stressors for a length of time extending between 6 and 28 days.<sup>23,44,45</sup> Types of stressors utilized for this method include restraint or tail-suspension stress, disruption of the light/dark cycle, cage tilt, food or water restriction, changing of cage mates, temperature reductions, exposure to soiled or wet bedding and random foot-shocks.<sup>45</sup> The idea behind the test is that mild stressors presented in an unpredictable manner over time induce a depression-like state. Accordingly, animals exposed to CMS show anhedonic behaviours, such as decreased sucrose consumption, impairments in natural reward associations (conditioned place preference) and responsiveness to brain stimulation.<sup>23</sup> They also show deficits in grooming, sexual behaviour and immune function.<sup>46</sup> Many of these effects can be reversed with chronic antidepressant exposure, but are not responsive to acute treatments. Also, abbreviated CMS models can induce depression-like behaviours in females, but not males, suggesting that this model is ideal for understanding biological differences in stress responses between males and females.<sup>44</sup> Although CMS can be time consuming and labour intensive, this model has powerful face, construct and predictive validity. The utility of CMS to understand the neurobiology of depression in both males and females makes it a very useful tool to test new compounds for the treatment of depression.

Repeated social defeat stress is another model of depression with strong face, construct and predictive validity. In this model, intruder mice experience a chronic physical social stress when placed into the home cage of a new larger aggressive conspecific each day for 10 days.<sup>47,48</sup> After the physical interaction, a divider is placed in the cage to separate the animals, but allows them to experience sensory cues through perforations in the partition. At the end of 10 days, the intruder mice are tested to see how much time they spend near a novel caged aggressor mouse in a new environment. Approximately two-thirds of the mice exhibit social avoidance behaviour and are considered susceptible, while the remaining one-third do not show avoidance behaviour and are considered resilient. Interestingly, both susceptible and resilient mice exhibit significant anxiety phenotypes, yet only susceptible mice show depression-like phenotypes measured by social avoidance, anhedonia, disruptions of the circadian system, metabolic changes and weight gain.<sup>49</sup> Some of these effects, including the social avoidance behaviour, can be reversed with chronic, but not acute, antidepressant treatment.<sup>47,50</sup> One of the benefits of this model is that it can separate animals that are vulnerable to the effects of stress from those who are not, which is consistent with human populations exposed to stress. Identifying populations of susceptible and resilient mice allows us to understand the underlying genetic and epigenetic differences that influence vulnerability to depression and anxiety. While social defeat is clearly a powerful model of depression, one of the major limitations of this type of stress is that it is difficult to test in female mice. Female mice are not naturally inclined to attack each other and males will not attack females. A few researchers have managed to examine the effects of repeated social defeat stress in females by using a rodent that is monogamous and territorial (California mouse; *Peromyscus*



*californicus*)<sup>51</sup> or by using nursing dams.<sup>52</sup> Studies of social defeat in females have shown similar effects as in males in measures such as anhedonia, conditioned place preference and metabolic effects. However, more research is clearly necessary to determine whether the same molecular cascades and neural circuitry are affected in both sexes.

As mentioned above, there is a large degree of overlap between anxiety and depressive disorders. Not surprisingly, chronic stress increases anxiety behaviour on the elevated plus maze, 0 maze and light–dark test. The psychology behind these tests is that the animal's drive to investigate a novel setting is in conflict with its fear of the novel environment and the potential for exposure.<sup>53</sup> However, it is unclear whether these tests are valid measures of anxiety in humans. While they are clearly alleviated acutely by benzodiazepine treatment, there is no evidence that they respond to chronic antidepressant treatment, and therefore lack the predictive validity necessary to test new compounds. Thus, in the future we will need to develop new animal behavioural models that mimic anxiety disorders in humans with far greater face, construct and predictive validity.

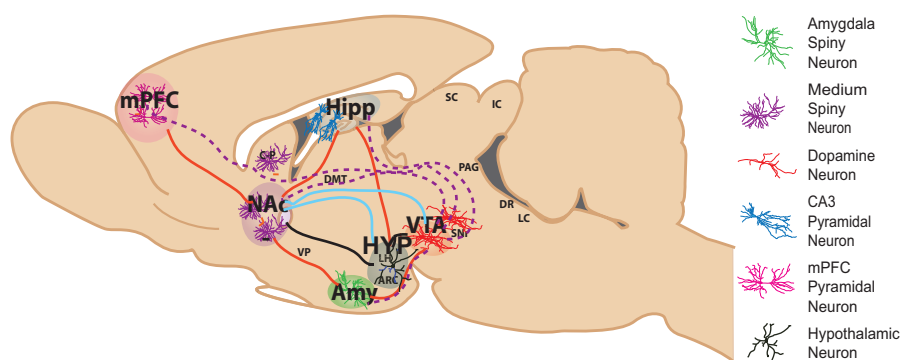
### 7.3.2 Acute Stress

The original pharmacological animal models of mood and anxiety disorders, including reserpine and  $\alpha$  methyl dopa exposure, were not able to discriminate the antidepressant properties of drugs such as tricyclics and MAOIs, even though these drug classes were already known to have therapeutic effects in humans. Reserpine induces a depression-like response by depleting catecholamines and serotonin rendering drugs that increased reuptake of these neurotransmitters ineffective.<sup>24</sup> The forced-swim test (FST) was introduced as the first animal model of depression that could be used as a screening device for potential new treatments. In the original design, rats were placed in a cylinder containing 15 cm of room-temperature water. They were given a 15-minute exposure to the water and then a 5-minute test of immobility 24 hours later.<sup>54</sup> The concept behind the test is that animals with higher levels of emotional despair would give up struggling sooner and remain immobile. This test was able to detect effects of tricyclics, MAOIs and atypical antidepressants, all of which decreased immobility in a dose-dependent manner. Later variations on this test were able to differentiate between different types of antidepressants. Rodents given drugs acting primarily on the NE system engaged in higher levels of climbing behaviour, whereas drugs associated with changes in the 5-HT system increased their swimming behaviour.<sup>55</sup> A dry version of the test, known as the tail-suspension test (TST), was also developed for use in mice to prevent hypothermia and increase repeatability.<sup>56</sup> Mice were suspended by their tails for 6 minutes and the amount of time spent immobile was recorded as a behavioural measure of despair.<sup>56</sup> The benefit of these types of tests is that they are rapid and allow for high-throughput screening of new compounds. In addition, they are fairly accurate at detecting the efficacy of current antidepressants. However, they lack predictive validity in that acute antidepressants effectively decrease the time spent immobile in both the FST and

TST, even though chronic treatment is necessary to alleviate depression symptoms in humans. In general, these types of tests work well for determining antidepressant efficacy and screening potential new drugs based on the monoamine hypothesis, but their validity as a model for depression or use in testing novel mechanisms of action is highly questionable.

## 7.4 Brain Regions Implicated in Depression and Anxiety Disorders

Depression and anxiety are complex disorders, which affect multiple brain regions (Figure 7.1), along with the endocrine, metabolic and inflammatory systems of the body. It is not yet clear to what extent the interplay between these systems and the brain controls the development and experience of these



**Figure 7.1** Interconnections of brain circuitry implicated in depression and anxiety disorders. A number of brain structures are thought to be involved in the etiology and symptoms of depression and anxiety disorders. The red solid lines represent excitatory glutamatergic afferents to the nucleus accumbens (NAc) from medial prefrontal cortex (mPFC), amygdala (Amy) and hippocampus (Hipp) and glutamatergic innervation of the hypothalamus (HYP) and ventral tegmental area (VTA) by the Amy and Hipp. GABAergic afferents, shown in blue, are inhibitory circuits. Inhibitory connections between the NAc and the VTA provide feedback to VTA dopamine neurons, whereas inhibitory circuits between the NAc and HYP may modulate the effects of stress on reward seeking. Dopamine neurons (shown in broken purple lines) project from the ventral tegmental area (VTA) and impinge directly on NAc, mPFC, Amy and Hipp neurons involved with the processing of hedonic stimuli. Peptidergic pathways through which the HYP alters neurotransmission in the NAc and VTA are shown in solid black lines. Each structure contains specialized neuronal cell types thought to play an integral role in the complex behavioural phenotypes associated with depression and anxiety. These cell types, colour-coded in the key, include Amy (green) and NAc (purple) spiny neurons, PFC (pink) and Hipp CA3 (blue) pyramidal neurons and HYP (black) and VTA dopamine neurons (red). Adapted with permission from ref. 165.

disorders, but a number of brain structures and biological pathways have been implicated. Initially it was thought that there was a limbic system of interconnected brain structures involved in the processing of emotion and memory,<sup>57</sup> including the hippocampus, septum, amygdala, anterior thalamic nuclei, limbic cortex and fornix. Over time, we have gained greater insight into the brain structures involved in the processing of emotional stimulus. As the technology used to understand brain function has progressed, more structures were added and theories modified. This section will provide an overview of some of the major structures empirically shown to be involved with the etiology and symptoms of depression and anxiety.

### 7.4.1 Hypothalamus

The hypothalamus is considered to be the centre of endocrine function in the central nervous system. The cells responsible for releasing hormones to stimulate gonadal and stress hormone synthesis, along with regulation of dark/light cycle and food intake, all have cell bodies in the hypothalamus. While all of these hormonal processes can be altered in depression and anxiety disorders, the stress pathway is thought to be involved with the etiology and symptoms. Stress induces an increase in corticotrophin-releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus.<sup>58</sup> CRH travels through the median eminence and induces an increase in adrenocortical-releasing hormone (ACTH) in the pituitary gland, which leads to release of glucocorticoids from the adrenal gland. Glucocorticoids then pass through the blood-brain barrier and bind to receptors in the hippocampus and frontal cortex that feed back onto the hypothalamus. In addition, glucocorticoids can act on CRH and ACTH directly to reduce hormonal output.<sup>59</sup> Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis occurs in half the population suffering from depression,<sup>60,61</sup> which is why tests of cortisol feedback have recently been used to diagnose depression.<sup>62</sup> As previously mentioned, most animal models of depression and anxiety use stress to induce a depression-like state associated with disruptions of HPA axis function, including changes in glucocorticoid release, negative feedback and CRH expression.<sup>63</sup>

CRH from the hypothalamus can also act on other neuronal structures to contribute to depression and anxiety. CRH can alter the NE arousal system through its interactions with the LC.<sup>64</sup> This pathway may contribute to sex differences in the occurrence of these disorders, since there are sex differences in the effects of stress on receptor internalization and electrophysiological sensitization in LC.<sup>65</sup> Additionally CRH and glucocorticoids interact with the immune system *via* interleukin-6 expressing neurons in the hypothalamus, which likely contribute to changes in inflammatory responses associated with these disorders.<sup>66</sup> In addition, the hypothalamus has myriad projections and inputs to and from other areas of the brain implicated in the stress response and symptoms of depression. These include, but are not limited to, direct and indirect connections to the hippocampus, nucleus accumbens and amygdala. As many of these regions have been implicated in depression, as discussed in

detail below, future studies to define this neural circuitry from the hypothalamus will provide us with important mechanistic insight into depression.

### **7.4.2 Hippocampus**

The hippocampus is traditionally considered a brain structure involved in learning and memory; however, it also has a large number of glucocorticoid and mineralocorticoid receptors<sup>67</sup> and has many connections to other structures involved with emotional processing. Animal research has indicated that various forms of hippocampal plasticity are affected by stress and are responsive to antidepressant treatment.<sup>68–70</sup> The hippocampus is also a part of a negative feedback loop with the HPA axis and the amygdala, so hippocampal dysfunction can result in widespread dysregulation of stress responses.

Imaging studies in humans indicate that people suffering from depression have reduced hippocampal volume.<sup>71–73</sup> It was proposed that this volumetric decrease is the result of a stress-induced decrease in neurotrophins, which reduce neurogenesis and overall hippocampal plasticity. This effect is partly reversed by antidepressant treatment.<sup>74–77</sup> The stress-induced decrease in plasticity may relate to the decrease in cognitive abilities of depressed individuals.<sup>78</sup> In fact, some have argued that decreased hippocampal function results in a decreased awareness of context and episodic memory, resulting in a generalization of negative experience.<sup>79</sup> Consistent with these human findings, animal studies have shown that stress reduces levels of the neurotrophin, brain-derived neurotrophic factor (BDNF),<sup>80</sup> which in turn decreases neurogenesis in the dentate gyrus as well as spine density<sup>81</sup> and dendritic complexity<sup>82</sup> in areas CA1 and CA3, respectively. Additionally, stress has been shown to alter long-term potentiation (LTP) and long-term depression (LTD), electrophysiological indicators of plasticity, suggesting that cellular function is altered by stress.<sup>83</sup> However, the majority of the evidence for a role of the hippocampus in depression comes from studies of the relationship between neurotrophin levels, neurogenesis and antidepressants. Antidepressants increase BDNF levels<sup>84</sup> and reverse stress-induced decreases in BDNF levels<sup>80</sup> and neurogenesis.<sup>36,37</sup> Because antidepressants do not have immediate effects on the symptoms of depression, the neurotrophin/neurogenesis theory of depression would explain this time lag as being due to the period needed for the incorporation and maturation of newly arisen granule cells.<sup>74,85</sup> Indeed, recent behavioural evidence indicates that neurogenesis is necessary for the therapeutic effects of SSRI treatment on depression-like behaviour.<sup>86</sup> That said, the majority of studies examining the role of neurogenesis in depression are performed in rodents, who have a much higher rate of cell proliferation than primates.<sup>87–89</sup> Furthermore, within rodents, stress-induced decreases in neurogenesis do not result in an overall decrease in hippocampal volume,<sup>36,37</sup> indicating that other mechanisms contribute to the decrease in hippocampal volume reported in patients suffering from depression. Finally, the neurotrophin/neurogenesis theory of depression suggests a causal relationship between decreased neurotrophins/neurogenesis and depression. Therefore, manipulations that decrease

trophic factors and neurogenesis in the hippocampus should induce depressive symptoms. The data show that while irradiation or BDNF knockout in the hippocampus severely reduce cell proliferation ( $\sim 85\%$  reduction), neither results in overt behavioural signs of depression.<sup>86</sup> Rather, both irradiation and BDNF knockout inhibit the antidepressant effects of SSRIs on depression-like behaviour. Together these studies suggest that both neurogenesis and neurotrophins may have a more prominent role in antidepressant response than in the etiology of depression.

### 7.4.3 Prefrontal Cortex

The prefrontal cortex has traditionally been thought of as an area tied to executive function. It is involved with the control and processing of cognitive and emotional stimuli, and is crucial for decision-making and goal-oriented behaviour. At their core, depression and anxiety disorders are diseases of perception. A stimulus that is inconvenient or unpleasant for one person can be perceived as devastating or life threatening to a person suffering from depression or anxiety.<sup>90–92</sup> As the prefrontal cortex is highly involved in the modulation of response to perceived stimuli, it is likely that this region is involved in the etiology of mood and anxiety disorders.

Imaging studies in humans suffering from depression show that they have changes in blood flow to the prefrontal cortex (PFC) indicating alterations in the cellular metabolism and activity in the region.<sup>93,94</sup> Increasing activity in the prefrontal cortex through trans-magnetic stimulation or deep brain stimulation<sup>95</sup> has been successfully used to induce remission in some patients with depression, even those with treatment-resistant forms of depression.<sup>96</sup> In animals, optogenetic stimulation of the prefrontal with channelrhodopsin in mouse cortex is antidepressant in the chronic social defeat stress model.<sup>97</sup>

Structurally, depression induces changes in the PFC; patients with depression have smaller PFC volumes along with other brain areas associated with emotional experience to which the PFC projects.<sup>71</sup> Much like the hippocampus, stress leads to decreases in BDNF in the PFC,<sup>98</sup> whereas antidepressants increase BDNF levels.<sup>84</sup> Stress also reduces the complexity of dendrites and decreases spine density, while antidepressants tend to increase the expression of genes associated with synaptic plasticity, suggesting that they may reverse these effects.<sup>99</sup> While it is highly controversial as to whether neurogenesis occurs in the frontal cortex,<sup>100–103</sup> it is thought that a small group of interneurons may arise from oligodendrocyte precursor cells.<sup>103</sup> *Post mortem* studies have shown that depressed humans have reduced cell numbers and atrophy within the PFC.<sup>104</sup> Translational studies in mice show that antidepressant treatment can increase the number of proliferating cells in the PFC and lead to an overall increase in the number of new cells that survive to maturity.<sup>105</sup> Additionally, *post mortem* tissue from depressed humans showed a reduction in immediate early genes (IEG) in the PFC, indicating a reduction in functional activity.<sup>97</sup> This result was mirrored within the same study by a reduction in IEG expression in susceptible mice exposed to repeated social

defeat. Together these studies suggest that depression and anxiety induce structural changes to the PFC and thus reduce its functional responsiveness and ability then to modulate the activity of target structures such as the hippocampus, amygdala and nucleus accumbens.

#### 7.4.4 Nucleus Accumbens

The nucleus accumbens (NAc) is a deep brain structure traditionally associated with the mesolimbic reward circuitry. Recent research has indicated a role for the NAc in the neurobiology of depression. It is thought that the NAc is involved with the reduction in hedonic experience associated with depression,<sup>106</sup> but recent studies of functionally altered gene expression in this region have also indicated a role for the NAc in other depression and anxiety associated behavioural tests, such as those induced by repeated social defeat stress.<sup>49,107–109</sup> To date, there has been little investigation in humans into structural and cellular changes in the NAc, although some studies of *post mortem* tissues indicate that there are epigenetic regulators of transcription in the NAc that are altered in depression.<sup>50,107</sup> Imaging studies have shown that people with depression have altered blood flow to the NAc,<sup>110</sup> and anhedonia, a key feature of depression, correlates with smaller NAc volume in healthy subjects.<sup>111</sup> Furthermore, depressed subjects have reduced functional responses within the NAc to monetary gains,<sup>112</sup> further highlighting the likelihood that reward experience is altered in people with depression through biological alterations in the NAc. Most importantly, deep brain electrical stimulation of the NAc results in remission in people with treatment-resistant depression,<sup>113</sup> and is antidepressant in animals models of depression.<sup>114</sup> These results suggest that this structure is a viable target to treat depression.

Stress studies in animals have indicated alterations in structural plasticity and cellular function in the NAc. Repeated social defeat stress increased the number of dendritic spines with smaller synapses and increased the baseline glutamate currents of medium spiny neurons in NAc in susceptible animals only.<sup>115</sup> Pre-natal stress also increases the density of dendritic spines (a site of excitatory connections) on neurons in the NAc,<sup>116</sup> and a number of studies have indicated changes in molecular cascades associated with neuroplasticity.<sup>44,49,50,108,115</sup> Interestingly, increases in BDNF, which would induce an antidepressant response in the hippocampus,<sup>117</sup> induce a depression-like phenotype in the NAc.<sup>118</sup> Likewise, reductions of BDNF in the NAc promote behavioural resiliency.<sup>119</sup> Together, these data indicate that changes in the NAc result in altered neuronal transmission and may interact with a variety of other structures with which the NAc has reciprocal connections, including the prefrontal cortex, hypothalamus, amygdala and hippocampus.<sup>6</sup>

#### 7.4.5 Amygdala

The amygdala controls the ability to associate stimuli with a frightening experience such as shock or predator exposure, and the neuroanatomical



circuitry involved with fear conditioning has been extensively studied (For review see reference 120). The changes that occur in the fear conditioning circuitry in the lateral amygdala<sup>121</sup> are well documented in anxiety disorders such as post-traumatic stress disorder (PTSD); however, more generally, the structure has become associated with negative emotional experience and may also be relevant in depression. Recently, there has been evidence that the amygdala is involved with the processing of positive emotional experience. Excitatory transmission from the basolateral amygdala (BLA) to the NAc, but not the prefrontal cortex to the NAc, increases reward-seeking behaviour.<sup>122</sup> This specificity suggests that the BLA inputs, in particular, may be involved in pathological reward processing in depression.

Imaging studies have indicated that people with depression have hyperactivation of the amygdala that correlates strongly with their depression severity.<sup>123</sup> Additionally, a meta analysis indicated that unmedicated depressed individuals have decreases in amygdala volume compared to healthy controls.<sup>124</sup> Imaging of activity in the Flanders sensitive line, a genetic rodent model of depression, showed increased activation of the amygdala and decreased activation of the prefrontal cortex compared to a control line (Flanders resistant) when exposed to a predator odour.<sup>125</sup> Given that there is also altered activation of the prefrontal cortex in humans suffering from depression,<sup>94</sup> it is likely that connectivity between the two structures is involved with the symptoms and possibly the etiology of mood disorders. This concept is supported by evidence that stress blocks BLA-dependent synaptic plasticity in the hippocampal/medial prefrontal cortex pathway.<sup>126</sup>

Functional alterations in amygdala activity may also contribute to anxiety disorders. Consistent with what is observed in depression, imaging studies in humans with social anxiety disorder also observe hyperactivation of the amygdala and reduced connectivity of the frontal cortex.<sup>127</sup> These changes in structural activity have also been shown in patients suffering from PTSD.<sup>128</sup> In mice, over-expression of corticotropin releasing hormone (CRH) in the central amygdala strongly enhances anxiety-like behaviours following application of an acute stressor, whereas viral knock-down of CRH attenuates the anxiety-like behaviours.<sup>129</sup> Additionally, optogenetic stimulation of pre-synaptic terminals projecting from the BLA to the central amygdala induces anxiolytic effects, whereas inhibition of this same connection induces an anxiogenic phenotype.<sup>130</sup>

Given the numerous changes occurring in multiple structures implicated in depression and anxiety disorders, it is clear that no single structure and no single neurotransmitter system is responsible for the etiology of these disorders. Rather, the data support a hypothesis that postulates that depression and anxiety disorders emerge from dysregulation of multiple overlapping systems and an imbalance in function of multiple structures. It is also becoming clear that a simple up or down regulation of neuropeptides and transmitters, such as BDNF or glutamate, may not be the cause of these disorders; rather, their role depends heavily upon the brain structure and pre-synaptic terminal from which these structures are enervated.



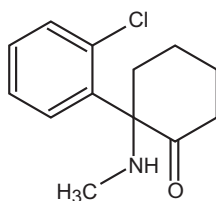
## 7.5 New Targets for Treatment of Anxiety and Mood Disorders

While many of the new drugs on the market are still permutations of the monoamine/catecholamine reuptake inhibitors, there is a new generation of antidepressants being developed and tested based on rational empirical data. While there are many novel strategies being developed, we chose to discuss three novel targets based on their promise and level of development.

### 7.5.1 Ketamine

Ketamine, an NMDA receptor antagonist, binds to the NMDA receptor with high affinity and prevents cellular damage by blocking excess calcium influx into the cell<sup>131</sup> (Figure 7.2). Ketamine has been used for years as a veterinary anaesthetic at high doses. However, a single low dose of ketamine has been shown rapidly to reduce (within 2 hours) depressive symptoms. The effects can be long lasting and the drug is highly effective in alleviating symptoms in a population of humans with treatment-resistant depression.<sup>132</sup> Ketamine treatment reduced symptoms in 71% of participants and caused full remission in 29%. One week later, 35% of the subjects still had a reduction in their symptoms. A recent study also showed that when ketamine is given in the emergency room to people with suicidal ideation, it can reduce suicidal thoughts within 40 minutes and depression rating scores within 4 hours.<sup>133</sup> Interestingly, symptoms of suicidal ideation are reduced for over 10 days from a single infusion of ketamine. Recent studies in animals show that ketamine can reverse the effects of CMS,<sup>134</sup> decrease immobility in the FST<sup>135</sup> and TST<sup>136</sup> and reduce escape failures in the LH model.<sup>136</sup>

As with the SSRI and tricyclic medications, the exact mechanisms that alleviate depression are still unknown. Unlike the SSRIs/tricyclics, ketamine was first used to treat depression because a large literature from preclinical studies indicated a role for NMDA receptor function in depression.<sup>137</sup> Ketamine is capable of increasing release of *in vivo* glutamate and dopamine in the PFC and also increases dopamine in the striatum.<sup>138</sup> Through these actions, ketamine can control synaptic plasticity of hippocampal and



**Figure 7.2** Chemical structure of ketamine. 2-(o-chlorophenyl)-2-(methylamino)cyclohexanone (hydrochloride).

prefrontal glutamatergic pyramidal neurons, a function mediated, in part, through a protein translation-dependent mechanism.<sup>135,139</sup> In addition to ketamine's effects at the NMDA receptor, it can also affect AMPA receptors. Pharmacological antagonists of AMPA receptors can prevent the anti-depressant effects of ketamine on behaviour,<sup>136</sup> suggesting that it may also have some agonistic effects. Ketamine is also known to act on dopamine and  $\mu$ -opioid receptors,<sup>140</sup> rapidly increase BDNF in the cortex<sup>135</sup> and inhibit a number of pro-inflammatory cytokines.<sup>141</sup> Ketamine's widespread effects on these well-established systems implicated in depression may explain its rapid effects and high remission rates across a heterogeneous population of depressed subjects.<sup>142</sup>

## 7.5.2 Cytokines

As mentioned earlier, depression acts on the immune system and alters pro-inflammatory cytokine expression. The "inflammatory and neurodegenerative" hypothesis of depression<sup>143</sup> postulates that depression and the neurodegeneration that accompanies it are, in part, caused by alterations in cytokine signalling and production within the body and brain. At the centre of this hypothesis is the idea that depression is an inflammatory disease.<sup>144</sup> First, there are higher rates of depression and anxiety disorders in patients with inflammatory diseases.<sup>145,146</sup> Furthermore, many cytokines are altered in depression and a meta-analysis found that interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNF- $\alpha$ ), in particular, are significantly elevated across studies of patients with depression.<sup>147</sup> Consistent with these findings, people who suffer from depression also have a higher incidence of heart disease, diabetes and arthritis,<sup>148</sup> conditions exacerbated by these inflammatory processes. Animal studies have also supported a functional role of cytokines in depression-associated behaviours. Brain and plasma levels of a variety of cytokines are increased following exposure to a number of stressors.<sup>149</sup> This alteration may be due, in part, to regulation of HPA axis stress hormones, since transgenic mice over-expressing IL-6 have dysfunctional HPA axis activity when exposed to a stressor.<sup>150</sup> Importantly, functional evidence shows that intra-cranial infusions of IL-6<sup>151</sup> or hippocampal infusions of interleukin-1 $\beta$  (IL-1 $\beta$ )<sup>152,153</sup> increase depression-associated behaviour and reduce neurogenesis, whereas infusion of an IL-1 $\beta$  antibody,<sup>153</sup> an IL-6 antibody<sup>151</sup> or an IL-6 knockout<sup>154</sup> blocks the depressive effects of CMS.

Currently a number of humanized mouse antibodies are being developed to target specific cytokines as new treatments for a variety of neuroinflammatory and auto-immune diseases. XOMA 052, a neutralizing monoclonal antibody for IL-1 $\beta$ , has been shown in preclinical studies to block the effects of acute inflammation and alter metabolic responses to chronic inflammation.<sup>155</sup> Tocilizumab, a humanized anti-IL-6 receptor antibody, is already being marketed in Japan as a treatment for Castleman disease and arthritis,<sup>156</sup> and is currently being considered for the treatment of unipolar and bipolar depression.<sup>157</sup> Ustekinumab, an antibody against interleukin 12/23, has already been shown to

decrease symptoms of depression and anxiety in humans treated for psoriasis.<sup>158</sup> Additionally, some of the more general anti-inflammatory drugs have shown promise in treating both depression and anxiety in humans. The cyclooxygenase-2 (COX-2) inhibitor rofecoxib significantly reduced co-morbid depression in an arthritis study.<sup>159</sup> Both animal models of depression and preliminary clinical trials of COX-2 inhibitors have produced promising results, although far more work is needed.<sup>160</sup> Given that these drugs have already been generated and, in some cases, FDA approved for the treatment of inflammatory diseases, clinical trials can now readily test their antidepressant properties.

### 7.5.3 Epigenetic Regulators of Depression

Epigenetics traditionally refers to the transfer of traits from one generation to another in the absence of changes in the genetic code.<sup>161</sup> Currently, it has also come to include alterations in enzyme modification of chromatin structure, which can alter access to DNA resulting in changes in transcription. Histones are part of a complex that makes up chromatin along with DNA and non-histone coding proteins. Through enzymatic modification of histones, the chromatin can exist in an open state, allowing transcription to occur, or can be condensed into a state that suppresses transcription.<sup>161</sup> Additionally, direct sites on the promoter regions of DNA can be methylated by enzymes known as DNA methyl-transferases (DNMTs) that lead to a condensed state of chromatin and suppression of transcription.<sup>108</sup> A new line of depression research is using animal models to understand the role of epigenetics in depression and anxiety disorders. Many of the enzymes involved in histone modification and gene suppression are altered in animal models of depression and in *post mortem* tissue from depressed subjects.<sup>50,108</sup> In the chronic social defeat stress models, some of the histone modifications are long lasting and can be found at least four weeks after the stress.<sup>162</sup> Furthermore, preclinical research has indicated a functional role for both histone modification and DNA methylation in stress-induced depression-like behaviour. For example, histone deacetylase (HDACs) inhibitors infused into the NAc can block the effects of repeated social stress on a variety of behavioural tests including social interaction, sucrose preference and the FST.<sup>50</sup> Additionally, viral-mediated over-expression of Dnmt3a in the NAc increases depression-like behaviour,<sup>108</sup> whereas Dnmt3a knockout blocks depression-like behaviours. Together, this growing body of preclinical research indicates that epigenetic regulators are a promising novel target for the future development of antidepressants and anxiolytic medications.

## 7.6 Conclusion: Creating Personalized Treatments for Anxiety and Depression

We are moving into a century in which tools for personalized medicine, such as genetic scans and biomarker identification, are being rapidly developed, and the possibility of using these approaches in practice is becoming a reality. Therefore, it is more important than ever that the medications we develop for

disorders such as depression and anxiety are based on rational targets that treat the underlying pathology. The reliance on the development of drugs based on the monoamine hypothesis of depression must change. Moreover, due to the overwhelming evidence that there are sex differences in the biological responses to stress, we need to include female subjects in preclinical drug-discovery efforts to take into account the numerous differences in pharmacokinetics<sup>163</sup> and pharmacodynamics<sup>164</sup> between the sexes. New drugs being developed must address the empirical evidence of a multi-systems view of the neurobiology of depression. Perhaps, if we can meet these criteria for drug development, we may be able to treat these debilitating disorders with greater efficacy and fewer deleterious sideeffects.

## References

1. R. C. Kessler, W. T. Chiu, O. Demler, K. R. Merikangas and E. E. Walters, *Arch. Gen. Psychiatry*, 2005, **62**, 617–627.
2. R. C. Kessler, K. A. McGonagle, M. Swartz, D. G. Blazer and C. B. Nelson, *J. Affect. Disord.*, 1993, **29**, 85–96.
3. M. Briley and C. Moret, *IDrugs*, 2000, **3**, 695–699.
4. L. Culpepper, *J. Clin. Psychiatry*, 2010, **71**(1), 4–9.
5. A. K. Beery and I. Zucker, *Neurosci. Biobehav. Rev.*, 2011, **35**, 565–572.
6. O. Berton and E. J. Nestler, *Nat. Rev. Neurosci.*, 2006, **7**, 137–151.
7. S. H. Preskorn, *J. Psychiatr. Pract.*, 2010, **16**, 334–339.
8. J. J. Schildkraut, *Am. J. Psychiatry*, 1965, **122**, 509–522.
9. M. A. Ellenbogen, S. N. Young, P. Dean, R. M. Palmour and C. Benkelfat, *Neuropsychopharmacology*, 1996, **15**, 465–474.
10. F. A. Moreno, C. A. McGahuey, M. P. Freeman and P. L. Delgado, *J. Clin. Psychiatry*, 2006, **67**, 1618–1623.
11. F. Martenyi, M. Dossenbach, K. Mraz and S. Metcalfe, *Eur. Neuro-psychopharmacol.*, 2001, **11**, 227–232.
12. P. L. Delgado and F. A. Moreno, *J. Clin. Psychiatry*, 2000, **61**(1), 5–12.
13. I. Hervas, C. M. Queiroz, A. Adell and F. Artigas, *Br. J. Pharmacol.*, 2000, **130**, 160–166.
14. P. Blier, C. de Montigny and Y. Chaput, *J. Clin. Psychopharmacol.*, 1987, **7**, 24S–35S.
15. S. Hjorth and S. B. Auerbach, *Neuropharmacology*, 1994, **33**, 331–334.
16. K. P. Lesch, *J. Affect. Disord.*, 2001, **62**, 57–76.
17. W. C. Drevets, E. Frank, J. C. Price, D. J. Kupfer, D. Holt, P. J. Greer, Y. Huang, C. Gautier and C. Mathis, *Biol. Psychiatry*, 1999, **46**, 1375–1387.
18. V. Klimek, J. Zak-Knapik and M. Mackowiak, *J. Psychiatry Neurosci.*, 1994, **19**, 63–67.
19. M. N. Subhash, B. N. Srinivas, K. Y. Vinod and S. Jagadeesh, *J. Neural Transm.*, 2000, **107**, 377–387.
20. A. W. Goddard, S. G. Ball, J. Martinez, M. J. Robinson, C. R. Yang, J. M. Russell and A. Shekhar, *Depress. Anxiety*, 2010, **27**, 339–350.

21. R. Mongeau, P. Blier and C. de Montigny, *Brain Res. Brain Res. Rev.*, 1997, **23**, 145–195.
22. P. Pacher and V. Kecskemeti, *Curr. Pharm. Des.*, 2004, **10**, 2463–2475.
23. C. Dalla, P. M. Pitychoutis, N. Kokras and Z. Papadopoulou-Daifoti, *Basic Clin. Pharmacol. Toxicol.*, 2010, **106**, 226–233.
24. W. T. McKinney, Jr and W. E. Bunney, Jr, *Arch. Gen. Psychiatry*, 1969, **21**, 240–248.
25. P. M. Plotsky and M. J. Meaney, *Brain Res. Mol. Brain Res.*, 1993, **18**, 195–200.
26. E. L. Kinnally, G. M. Karere, L. A. Lyons, S. P. Mendoza, W. A. Mason and J. P. Capitanio, *Dev. Psychopathol.*, 2010, **22**, 35–44.
27. L. A. Diehl, P. P. Silveira, M. C. Leite, L. M. Crema, A. K. Portella, M. N. Billodre, E. Nunes, T. P. Henriques, L. B. Fidelix-da-Silva, M. D. Heis, C. A. Goncalves, J. A. Quillfeldt and C. Dalmaz, *Brain Res.*, 2007, **1144**, 107–116.
28. J. B. Overmier and M. E. Seligman, *J. Comp. Physiol. Psychol.*, 1967, **63**, 28–33.
29. M. E. Seligman, *Annu. Rev. Med.*, 1972, **23**, 407–412.
30. M. E. Seligman, R. A. Rosellini and M. J. Kozak, *J. Comp. Physiol. Psychol.*, 1975, **88**, 542–547.
31. M. E. Seligman and G. Beagley, *J. Comp. Physiol. Psychol.*, 1975, **88**, 534–541.
32. B. Vollmayr, C. Simonis, S. Weber, P. Gass and F. Henn, *Biol. Psychiatry*, 2003, **54**, 1035–1040.
33. J. M. Weiss and H. I. Glazer, *Psychosom. Med.*, 1975, **37**, 499–521.
34. J. M. Weiss, E. A. Stone and N. Harrell, *J. Comp. Physiol. Psychol.*, 1970, **72**, 153–160.
35. A. Zazpe, I. Artaiz, L. Labeaga, M. L. Lucero and A. Orjales, *Neuropharmacology*, 2007, **52**, 975–984.
36. H. Chen, G. N. Pandey and Y. Dwivedi, *NeuroReport*, 2006, **17**, 863–867.
37. J. E. Malberg and R. S. Duman, *Neuropsychopharmacology*, 2003, **28**, 1562–1571.
38. J. A. Jenkins, P. Williams, G. L. Kramer, L. L. Davis and F. Petty, *Biol. Psychol.*, 2001, **58**, 147–158.
39. H. L. Steenbergen, R. P. Heinsbroek, A. Van Hest and N. E. Van de Poll, *Physiol. Behav.*, 1990, **48**, 571–576.
40. T. J. Shors, J. Mathew, H. M. Sisti, C. Edgecomb, S. Beckoff and C. Dalla, *Biol. Psychiatry*, 2007, **62**, 487–495.
41. C. Dalla, C. Edgecomb, A. S. Whetstone and T. J. Shors, *Neuropsychopharmacology*, 2008, **33**, 1559–1569.
42. H. L. Steenbergen, R. P. Heinsbroek, F. Van Haaren and N. E. Van de Poll, *Physiol. Behav.*, 1989, **45**, 781–787.
43. A. D. Sherman, J. L. Sacquitne and F. Petty, *Pharmacol. Biochem. Behav.*, 1982, **16**, 449–454.
44. Q. LaPlant, S. Chakravarty, V. Vialou, S. Mukherjee, J. W. Koo, G. Kalahasti, K. R. Bradbury, S. V. Taylor, I. Maze, A. Kumar, A. Graham,

- S. G. Birnbaum, V. Krishnan, H. T. Truong, R. L. Neve, E. J. Nestler and S. J. Russo, *Biol. Psychiatry*, 2009, **65**, 874–880.
45. P. Willner, *Psychopharmacology (Berl.)*, 1997, **134**, 319–329.
46. V. Krishnan, O. Berton and E. Nestler, *Am. J. Psychiatry*, 2008, **165**, 1109.
47. V. Zachariou, C. A. Bolanos, D. E. Selley, D. Theobald, M. P. Cassidy, M. B. Kelz, T. Shaw-Lutchman, O. Berton, L. J. Sim-Selley, R. J. Dileone, A. Kumar and E. J. Nestler, *Nat. Neurosci.*, 2006, **9**, 205–211.
48. S. A. Golden, H. E. Covington, 3rd, O. Berton and S. J. Russo, *Nat. Protoc.*, 2011, **6**, 1183–1191.
49. V. Krishnan, M. H. Han, D. L. Graham, O. Berton, W. Renthal, S. J. Russo, Q. Laplant, A. Graham, M. Lutter, D. C. Lagace, S. Ghose, R. Reister, P. Tannous, T. A. Green, R. L. Neve, S. Chakravarty, A. Kumar, A. J. Eisch, D. W. Self, F. S. Lee, C. A. Tamminga, D. C. Cooper, H. K. Gershenfeld and E. J. Nestler, *Cell*, 2007, **131**, 391–404.
50. H. E. Covington, 3rd, I. Maze, Q. C. LaPlant, V. F. Vialou, Y. N. Ohnishi, O. Berton, D. M. Fass, W. Renthal, A. J. Rush, 3rd, E. Y. Wu, S. Ghose, V. Krishnan, S. J. Russo, C. Tamminga, S. J. Haggarty and E. J. Nestler, *J. Neurosci.*, 2009, **29**, 11451–11460.
51. B. C. Trainor, M. C. Pride, R. Villalon Landeros, N. W. Knoblauch, E. Y. Takahashi, A. L. Silva and K. K. Crean, *PLoS One*, 2011, **6**, e17405.
52. A. Shimamoto, J. F. Debold, E. N. Holly and K. A. Miczek, *Psychopharmacology (Berl.)*, 2011, **218**(1), 271.
53. J. F. Cryan and A. Holmes, *Nat. Rev. Drug Discov.*, 2005, **4**, 775–790.
54. R. D. Porsolt, G. Anton, N. Blavet and M. Jalfre, *Eur. J. Pharmacol.*, 1978, **47**, 379–391.
55. M. J. Detke and I. Lucki, *Behav. Brain Res.*, 1996, **73**, 43–46.
56. L. Steru, R. Chermat, B. Thierry and P. Simon, *Psychopharmacology (Berl.)*, 1985, **85**, 367–370.
57. L. P. Mac, *Psychosom. Med.*, 1949, **11**, 338–353.
58. A. M. Bao, G. Meynen and D. F. Swaab, *Brain Res. Rev.*, 2008, **57**, 531–553.
59. C. Osterlund and R. L. Spencer, *J. Endocrinol.*, 2011, **208**, 311–322.
60. C. F. Gillespie and C. B. Nemeroff, *Psychosom. Med.*, 2005, **67**(1), S26–S28.
61. P. W. Gold and G. P. Chrousos, *Mol. Psychiatry*, 2002, **7**, 254–275.
62. H. Kunugi, H. Hori, N. Adachi and T. Numakawa, *Psychiatry Clin. Neurosci.*, 2010, **64**, 447–459.
63. S. Checkley, *Br. Med. Bull.*, 1996, **52**, 597–617.
64. R. J. Valentino, B. Reyes, E. Van Bockstaele and D. Bangasser, *Neuropharmacology*, 2012, **62**(1), 13.
65. D. A. Bangasser, A. Curtis, B. A. Reyes, T. T. Bethea, I. Parastatidis, H. Ischiropoulos, E. J. Van Bockstaele and R. J. Valentino, *Mol. Psychiatry*, 2010, **15**(877), 896–904.
66. K. Kageyama and T. Suda, *Endocr. J.*, 2009, **56**, 335–344.
67. R. Jankord and J. P. Herman, *Ann. NY Acad. Sci.*, 2008, **1148**, 64–73.

68. R. S. Duman, G. R. Heninger and E. J. Nestler, *Arch. Gen. Psychiatry*, 1997, **54**, 597–606.
69. R. S. Duman, *Biol. Psychiatry*, 2004, **56**, 140–145.
70. J. E. Malberg, A. J. Eisch, E. J. Nestler and R. S. Duman, *J. Neurosci.*, 2000, **20**, 9104–9110.
71. D. Arnone, A. M. McIntosh, K. P. Ebmeier, M. R. Munafo and I. M. Anderson, *Eur. Neuropsychopharmacol.*, 2012, **22**(1), 1.
72. Y. I. Sheline, P. W. Wang, M. H. Gado, J. G. Csernansky and M. W. Vannier, *Proc. Natl Acad. Sci. USA*, 1996, **93**, 3908–3913.
73. J. Cole, S. G. Costafreda, P. McGuffin and C. H. Fu, *J. Affect. Disord.*, 2011, **134**(1–3), 483.
74. R. M. Sapolsky, *Biol. Psychiatry*, 2004, **56**, 137–139.
75. J. Chen, S. S. Newton, L. Zeng, D. H. Adams, A. L. Dow, T. M. Madsen, E. J. Nestler and R. S. Duman, *Neuropsychopharmacology*, 2004, **29**, 23–31.
76. B. L. Jacobs, H. van Praag and F. H. Gage, *Mol. Psychiatry*, 2000, **5**, 262–269.
77. B. L. Jacobs, *Brain Behav. Immun.*, 2002, **16**, 602–609.
78. M. Vythilingam, E. Vermetten, G. M. Anderson, D. Luckenbaugh, E. R. Anderson, J. Snow, L. H. Staib, D. S. Charney and J. D. Bremner, *Biol. Psychiatry*, 2004, **56**, 101–112.
79. S. Becker and J. M. Wojtowicz, *Trends Cogn. Sci.*, 2007, **11**, 70–76.
80. H. D. Schmidt and R. S. Duman, *Behav. Pharmacol.*, 2007, **18**, 391–418.
81. T. J. Shors, J. Falduto and B. Leuner, *Eur. J. Neurosci.*, 2004, **19**, 145–150.
82. A. M. Magarinos, A. Deslandes and B. S. McEwen, *Eur. J. Pharmacol.*, 1999, **371**, 113–122.
83. J. G. Howland and Y. T. Wang, *Prog. Brain Res.*, 2008, **169**, 145–158.
84. D. T. Balu, G. E. Hodes, B. T. Anderson and I. Lucki, *Neuropsychopharmacology*, 2009, **34**, 1764–1773.
85. H. van Praag, A. F. Schinder, B. R. Christie, N. Toni, T. D. Palmer and F. H. Gage, *Nature*, 2002, **415**, 1030–1034.
86. L. Santarelli, M. Saxe, C. Gross, A. Surget, F. Battaglia, S. Dulawa, N. Weisstaub, J. Lee, R. Duman, O. Arancio, C. Belzung and R. Hen, *Science*, 2003, **301**, 805–809.
87. H. A. Cameron and R. D. McKay, *J. Comp. Neurol.*, 2001, **435**, 406–417.
88. D. R. Kornack and P. Rakic, *Proc. Natl Acad. Sci. USA*, 1999, **96**, 5768–5773.
89. M. S. Rao and A. K. Shetty, *Eur. J. Neurosci.*, 2004, **19**, 234–246.
90. L. A. Stapinski, M. J. Abbott and R. M. Rapee, *Behav. Res. Ther.*, 2010, **48**, 1097–1104.
91. C. G. Beevers, P. Clasen, E. Stice and D. Schnyer, *Neuroscience*, 2010, **167**, 97–103.
92. S. R. Chamberlain and B. J. Sahakian, *Curr. Psychiatry Rep.*, 2006, **8**, 458–463.
93. J. C. Soares and J. J. Mann, *J. Psychiatr. Res.*, 1997, **31**, 393–432.



94. M. Liotti and H. S. Mayberg, *J. Clin. Exp. Neuropsychol.*, 2001, **23**, 121–136.
95. H. Johansen-Berg, D. A. Gutman, T. E. Behrens, P. M. Matthews, M. F. Rushworth, E. Katz, A. M. Lozano and H. S. Mayberg, *Cereb. Cortex*, 2008, **18**, 1374–1383.
96. M. S. George and R. M. Post, *Am. J. Psychiatry*, 2011, **168**, 356–364.
97. H. E. Covington, 3rd, M. K. Lobo, I. Maze, V. Vialou, J. M. Hyman, S. Zaman, Q. LaPlant, E. Mouzon, S. Ghose, C. A. Tamminga, R. L. Neve, K. Deisseroth and E. J. Nestler, *J. Neurosci.*, 2010, **30**, 16082–16090.
98. F. Fumagalli, F. Bedogni, J. Perez, G. Racagni and M. A. Riva, *Eur. J. Neurosci.*, 2004, **20**, 1348–1354.
99. M. N. Alme, K. Wibrand, G. Dagestad and C. R. Bramham, *Neural. Plast.*, 2007, **2007**, 26496.
100. M. Kodama, T. Fujioka and R. S. Duman, *Biol. Psychiatry*, 2004, **56**, 570–580.
101. A. G. Dayer, K. M. Cleaver, T. Abouantoun and H. A. Cameron, *J. Cell Biol.*, 2005, **168**, 415–427.
102. B. Czeh, J. I. Muller-Keuker, R. Rygula, N. Abumaria, C. Hiemke, E. Domenici and E. Fuchs, *Neuropsychopharmacology*, 2007, **32**, 1490–1503.
103. H. A. Cameron and A. G. Dayer, *Biol. Psychiatry*, 2008, **63**, 650–655.
104. G. Rajkowska, *Biol. Psychiatry*, 2000, **48**, 766–777.
105. G. E. Hodes, T. E. Hill-Smith, R. F. Suckow, T. B. Cooper and I. Lucki, *J. Pharmacol. Exp. Ther.*, 2010, **332**, 266–273.
106. E. J. Nestler, M. Barrot, R. J. DiLeone, A. J. Eisch, S. J. Gold and L. M. Monteggia, *Neuron*, 2002, **34**, 13–25.
107. M. B. Wilkinson, C. Dias, J. Magida, M. Mazei-Robison, M. Lobo, P. Kennedy, D. Dietz, H. Covington, 3rd, S. Russo, R. Neve, S. Ghose, C. Tamminga and E. J. Nestler, *J. Neurosci.*, 2011, **31**, 9084–9092.
108. Q. LaPlant, V. Vialou, H. E. Covington, 3rd, D. Dumitriu, J. Feng, B. L. Warren, I. Maze, D. M. Dietz, E. L. Watts, S. D. Iniguez, J. W. Koo, E. Mouzon, W. Renthall, F. Hollis, H. Wang, M. A. Noonan, Y. Ren, A. J. Eisch, C. A. Bolanos, M. Kabbaj, G. Xiao, R. L. Neve, Y. L. Hurd, R. S. Oosting, G. Fan, J. H. Morrison and E. J. Nestler, *Nat. Neurosci.*, 2010, **13**, 1137–1143.
109. M. Barrot, J. D. Olivier, L. I. Perrotti, R. J. DiLeone, O. Berton, A. J. Eisch, S. Impey, D. R. Storm, R. L. Neve, J. C. Yin, V. Zachariou and E. J. Nestler, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 11435–11440.
110. W. C. Drevets, *Curr. Opin. Neurobiol.*, 2001, **11**, 240–249.
111. J. Wacker, D. G. Dillon and D. A. Pizzagalli, *Neuroimage*, 2009, **46**, 327–337.
112. D. A. Pizzagalli, A. J. Holmes, D. G. Dillon, E. L. Goetz, J. L. Birk, R. Bogdan, D. D. Dougherty, D. V. Iosifescu, S. L. Rauch and M. Fava, *Am. J. Psychiatry*, 2009, **166**, 702–710.
113. B. H. Bewernick, R. Hurlmann, A. Matusch, S. Kayser, C. Grubert, B. Hadrysiewicz, N. Axmacher, M. Lemke, D. Cooper-Mahkorn, M. X.

- Cohen, H. Brockmann, D. Lenartz, V. Sturm and T. E. Schlaepfer, *Biol. Psychiatry*, 2010, **67**, 110–116.
114. S. M. Falowski, A. Sharan, B. A. Reyes, C. Sikkema, P. Szot and E. J. Van Bockstaele, *Neurosurgery*, 2011, **69**(6), 1281.
115. D. J. Christoffel, S. A. Golden, D. Dumitriu, A. J. Robison, W. G. Janssen, H. F. Ahn, V. Krishnan, C. M. Reyes, M. H. Han, J. L. Ables, A. J. Eisch, D. M. Dietz, D. Ferguson, R. L. Neve, P. Greengard, Y. Kim, J. H. Morrison and S. J. Russo, *J. Neurosci.*, 2011, **31**, 314–321.
116. A. Muhammad and B. Kolb, *Dev. Neurosci.*, 2011, **33**(2), 85.
117. Y. Shirayama, A. C. Chen, S. Nakagawa, D. S. Russell and R. S. Duman, *J. Neurosci.*, 2002, **22**, 3251–3261.
118. A. J. Eisch, C. A. Bolanos, J. de Wit, R. D. Simonak, C. M. Pudiak, M. Barrot, J. Verhaagen and E. J. Nestler, *Biol. Psychiatry*, 2003, **54**, 994–1005.
119. O. Berton, C. A. McClung, R. J. Dileone, V. Krishnan, W. Renthal, S. J. Russo, D. Graham, N. M. Tsankova, C. A. Bolanos, M. Rios, L. M. Monteggia, D. W. Self and E. J. Nestler, *Science*, 2006, **311**, 864–868.
120. J. E. LeDoux, *Annu. Rev. Neurosci.*, 2000, **23**, 155–184.
121. J. J. Radley, L. R. Johnson, W. G. Janssen, J. Martino, R. Lamprecht, P. R. Hof, J. E. LeDoux and J. H. Morrison, *Eur. J. Neurosci.*, 2006, **24**, 876–884.
122. G. D. Stuber, D. R. Sparta, A. M. Stamatakis, W. A. van Leeuwen, J. E. Hardjoprajitno, S. Cho, K. M. Tye, K. A. Kempadoo, F. Zhang, K. Deisseroth and A. Bonci, *Nature*, 2011, **475**, 377–380.
123. M. A. Peluso, D. C. Glahn, K. Matsuo, E. S. Monkul, P. Najt, F. Zamarrripa, J. Li, J. L. Lancaster, P. T. Fox, J. H. Gao and J. C. Soares, *Psychiatry Res.*, 2009, **173**, 158–161.
124. J. P. Hamilton, M. Siemer and I. H. Gotlib, *Mol. Psychiatry*, 2008, **13**, 993–1000.
125. W. Huang, M. E. Heffernan, Z. Li, N. Zhang, D. H. Overstreet and J. A. King, *Neurosci. Lett.*, 2011, **489**, 74–78.
126. G. Richter-Levin and M. Maroun, *Cereb. Cortex*, 2010, **20**, 2433–2441.
127. A. Hahn, P. Stein, C. Windischberger, A. Weissenbacher, C. Spindelegger, E. Moser, S. Kasper and R. Lanzenberger, *Neuroimage*, 2011, **56**, 881–889.
128. M. Koenigs and J. Grafman, *Neuroscientist*, 2009, **15**, 540–548.
129. L. Regev, M. Tsoory, S. Gil and A. Chen, *Biol. Psychiatry*, 2012, **71**(4), 317.
130. K. M. Tye, R. Prakash, S. Y. Kim, L. E. Fenno, L. Grosenick, H. Zarabi, K. R. Thompson, V. Gradinaru, C. Ramakrishnan and K. Deisseroth, *Nature*, 2011, **471**, 358–362.
131. C. Zarate, Jr, R. Machado-Vieira, I. Henter, L. Ibrahim, N. Diazgranados and G. Salvatore, *Harv. Rev. Psychiatry*, 2010, **18**, 293–303.
132. C. A. Zarate, Jr, J. B. Singh, P. J. Carlson, N. E. Brutsche, R. Ameli, D. A. Luckenbaugh, D. S. Charney and H. K. Manji, *Arch. Gen. Psychiatry*, 2006, **63**, 856–864.

133. G. L. Larkin and A. L. Beautrais, *Int. J. Neuropsychopharmacol.*, 2011, **8**, 1127–1131.
134. L. S. Garcia, C. M. Comim, S. S. Valvassori, G. Z. Reus, L. Stertz, F. Kapczinski, E. C. Gavioli and J. Quevedo, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2009, **33**, 450–455.
135. A. E. Autry, M. Adachi, E. Nosyreva, E. S. Na, M. F. Los, P. F. Cheng, E. T. Kavalali and L. M. Monteggia, *Nature*, 2011, **475**, 91–95.
136. H. Koike, M. Iijima and S. Chaki, *Behav. Brain Res.*, 2011, **224**, 107–111.
137. R. M. Berman, A. Cappiello, A. Anand, D. A. Oren, G. R. Heninger, D. S. Charney and J. H. Krystal, *Biol. Psychiatry*, 2000, **47**, 351–354.
138. B. Moghaddam, B. Adams, A. Verma and D. Daly, *J. Neurosci.*, 1997, **17**, 2921–2927.
139. N. Li, B. Lee, R. J. Liu, M. Banasr, J. M. Dwyer, M. Iwata, X. Y. Li, G. Aghajanian and R. S. Duman, *Science*, 2010, **329**, 959–964.
140. M. aan het Rot, K. A. Collins, J. W. Murrough, A. M. Perez, D. L. Reich, D. S. Charney and S. J. Mathew, *Biol. Psychiatry*, 2010, **67**, 139–145.
141. T. Taniguchi and K. Yamamoto, *Mini-Rev. Med. Chem.*, 2005, **5**, 241–245.
142. J. J. Yang, Z. Q. Zhou and C. Yang, *Psychol. Med.*, 2011, **41**, 1787–1789.
143. M. Maes, R. Yirmiya, J. Noraberg, S. Brene, J. Hibbeln, G. Perini, M. Kubera, P. Bob, B. Lerer and M. Maj, *Metab. Brain Dis.*, 2009, **24**, 27–53.
144. M. Maes, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2011, **35**, 664–675.
145. L. A. Graff, J. R. Walker and C. N. Bernstein, *Inflamm. Bowel Dis.*, 2009, **15**, 1105–1118.
146. M. Maes, M. Kubera, E. Obuchowiczwa, L. Goehler and J. Brzeszcz, *Neuroendocrinol Lett.*, 2011, **32**, 7–24.
147. Y. Dowlati, N. Herrmann, W. Swardfager, H. Liu, L. Sham, E. K. Reim and K. L. Lancot, *Biol. Psychiatry*, 2010, **67**, 446–457.
148. W. S. Fenton and E. S. Stover, *Curr. Opin. Psychiatry*, 2006, **19**, 421–427.
149. C. Song and H. Wang, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2011, **35**, 760–768.
150. J. Raber, R. D. O’shea, F. E. Bloom and I. L. Campbell, *J. Neurosci.*, 1997, **17**, 9473–9480.
151. M. W. Meagher, R. R. Johnson, E. E. Young, E. G. Vichaya, S. Lunt, E. A. Hardin, M. A. Connor and C. J. Welsh, *Brain Behav. Immun.*, 2007, **21**, 1083–1095.
152. J. W. Koo, S. J. Russo, D. Ferguson, E. J. Nestler and R. S. Duman, *Proc. Natl Acad. Sci. USA*, 2010, **107**, 2669–2674.
153. J. W. Koo and R. S. Duman, *Proc. Natl Acad. Sci. USA*, 2008, **105**, 751–756.
154. F. J. Monje, M. Cabatic, I. Divisch, E. J. Kim, K. R. Herkner, B. R. Binder and D. D. Pollak, *J. Neurosci.*, 2011, **31**, 9075–9083.
155. A. M. Owyang, H. Issafras, J. Corbin, K. Ahluwalia, P. Larsen, E. Pongo, M. Handa, A. H. Horwitz, M. K. Roell, M. Haak–Friendscho and L. Masat, *MAbs*, 2011, **3**, 49–60.
156. A. Venkiteshwaran, *MAbs*, 2009, **1**, 432–438.

157. E. Brietzke, M. Scheinberg and B. Lafer, *Med. Hypotheses*, 2011, **76**, 21–23.
158. R. G. Langley, S. R. Feldman, C. Han, B. Schenkel, P. Szapary, M. C. Hsu, J. P. Ortonne, K. B. Gordon and A. B. Kimball, *J. Am. Acad. Dermatol.*, 2010, **63**, 457–465.
159. E. Collantes-Estevez and C. Fernandez-Perez, *Curr. Med. Res. Opin.*, 2003, **19**, 402–410.
160. N. Muller, *Curr. Opin. Investig. Drugs*, 2010, **11**, 31–42.
161. N. Tsankova, W. Renthal, A. Kumar and E. J. Nestler, *Nat. Rev. Neurosci.*, 2007, **8**, 355–367.
162. N. M. Tsankova, O. Berton, W. Renthal, A. Kumar, R. L. Neve and E. J. Nestler, *Nat. Neurosci.*, 2006, **9**, 519–525.
163. N. Kokras, C. Dalla and Z. Papadopoulou-Daifoti, *Expert Opin. Drug Metab. Toxicol.*, 2011, **7**, 213–226.
164. I. Beierle, B. Meibohm and H. Derendorf, *Int. J. Clin. Pharmacol. Ther.*, 1999, **37**, 529–547.
165. S. J. Russo, M. S. Mazei-Robison, J. L. Ables and E. J. Nestler, *Neuropharmacology*, 2009, **56**(1), 73–82.

## CHAPTER 8

# *Beyond SSRIs: Second-generation Reuptake Inhibitors for the Treatment of Depression*

PETER T. GALLAGHER

Lilly Research Centre, Windlesham, Surrey, GU20 6PH, UK  
Email: Gallagher\_P@lilly.com

### **8.1 Profile and Limitations of Selective Serotonin Reuptake Inhibitors (SSRIs)**

Depression has been assessed by the World Health Organization (WHO) to affect 121 million people globally. The WHO has also reported that unipolar depression causes the greatest percentage of years lost due to disability (YLD). YLD is defined as the measure of the number of years of healthy life lost through time spent in states of less than full health.<sup>1</sup> Depression is more prevalent in women than in men and has a lifetime prevalence rate of 12.8%.<sup>2</sup> Severe depression may ultimately result in suicide, the incidence of which is 15% amongst the severely depressed.<sup>3</sup> A major depressive episode is characterized by a period of at least two weeks during which there is either depressed mood or the loss of interest/pleasure in nearly all activities (anhedonia). Additional symptoms include changes in appetite, altered psychomotor activity, disturbed sleep (insomnia or hypersomnia), decreased energy (fatigue or tiredness), feelings of worthlessness or guilt, difficulty thinking, concentrating or making decisions, recurrent thoughts of death and/or suicidal ideation, plans or attempts.

---

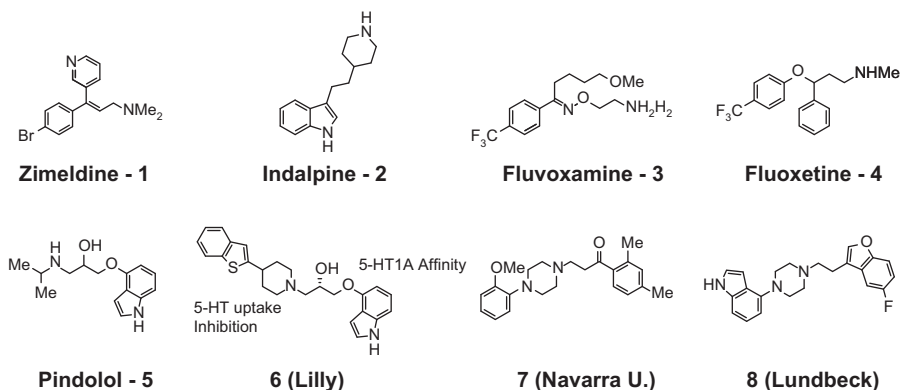
RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)



**Figure 8.1** Early SERT inhibitors, pindolol and exemplar serotonin reuptake inhibitor/5-HT<sub>1A</sub> antagonists.

Unsurprisingly there have been many attempts to treat depression and therapy has evolved from St John's Wort (*Hypericum*), through electro-convulsive therapy, monoamine oxidase inhibitors, tricyclic antidepressants and in the 1980s the introduction of Selective Serotonin Reuptake Inhibitors (SSRIs), *e.g.* zimeldine (1), indalpine (2), fluvoxamine (3) and fluoxetine (4) (Figure 8.1).

The SSRIs ushered in an era of safer antidepressant treatment as they offered a reduced side-effect profile compared to the standard of therapy prior to the SSRIs, the tricyclic antidepressants.<sup>4</sup> Tricyclic antidepressants, while effective, possessed a poly-pharmacological profile, which included muscarinic, histaminergic and adrenergic receptor interactions and this resulted in the well-recognized side-effects of dry mouth, blurred vision, constipation, urinary difficulties, tremor, lowering of blood pressure (resulting in postural hypotension), impotence, tachycardia and arrhythmia. Unlike SSRIs tricyclic antidepressants are not safe when taken in overdose, as fatal cardiotoxicity may result.

The safety profile and broad clinical acceptance of the SSRIs has led them to be tested in a wide range of syndromes including obsessive-compulsive disorder, premature ejaculation, pathological gambling, diabetic neuropathy, generalized anxiety disorder, agoraphobia, panic disorder and post-traumatic stress disorder with varying degrees of effectiveness.<sup>5–15</sup>

However, the SSRIs are not the ultimate solution to antidepressant therapy as they have notable limitations, the most significant of which is partial effectiveness, with a lack of response to treatment in 29–46% of the depressed population.<sup>16</sup> The second limiting feature that characterizes SSRIs is the delay in onset of antidepressant effectiveness, which is commonly between 3 and 4 weeks. Additionally the principal unwanted side-effects of SSRIs are sleep disturbance, nausea and sexual dysfunction, although the last of these side-effects has been utilized in newer treatments for premature ejaculation. Accordingly there has been a search for "augmented" SSRIs as well as attempts to identify the post-synaptic target of the serotonin (5-HT) released by the

SSRIs.<sup>17</sup> This chapter surveys approaches to augmented SSRIs as well as progress in targeting pre- and post-synaptic 5-HT receptors that have been implicated in depression and anxiety.

## 8.2 Serotonin Reuptake Inhibition Augmentation Strategies

Augmentation of SSRIs has evolved in two distinct ways. Firstly researchers have attempted to seek a preclinical rationalization of the mechanism of SSRI action and subsequently to target adjunct pre- and post-synaptic serotonin receptors. Secondly, following clinical observation atypical antipsychotics have been found to augment SSRI action and enhance their therapeutic effectiveness towards treatment-resistant depression.<sup>18</sup> The effectiveness of augmentation of SSRI therapy with atypical antipsychotics has led to preclinical rationalization of the mechanism of antipsychotic action and generated *de novo* Serotonin Reuptake Inhibitor (SRI) plus strategies.

### 8.2.1 Serotonin Reuptake Inhibition and 5-HT<sub>1A</sub> Antagonism

To understand the potential value of augmentation, it is necessary to consider the impact and time course of SSRI treatment alone. Following acute treatment with an SSRI, extra-cellular serotonin concentrations are raised through inhibition of the serotonin reuptake transporter; however, the acute increase in serotonin concentrations does not result in an immediate antidepressant action, which is delayed by 2–3 weeks.<sup>19</sup> In an attempt to rationalize the delay in onset of SSRI action De Montigny investigated the impact of zimeldine on rat dorsal raphe 5-HT neurons.<sup>20</sup> Zimeldine, in common with the other SSRIs, had demonstrated a delayed onset of antidepressant action.<sup>21</sup>

De Montigny showed that pre-treating rats with zimeldine resulted in time-dependent reduction in the firing of dorsal raphe neurons. After 2 days pre-treatment, neuronal firing was reduced by 90%, but following 7 days pre-treatment firing was reduced by 15% and after pre-treatment with zimeldine for 14 days, firing reached control levels. The initial down-regulation and the delayed re-establishment of firing were proposed as the underlying mechanism responsible for the delayed onset of action of zimeldine and other SSRIs. The receptor subsequently identified as responding to the increased serotonin concentration and that mediates reduced neuronal firing is the pre-synaptic 5-HT<sub>1A</sub> auto-receptor.<sup>22</sup> The conclusion from these studies is that the acute elevation of serotonin concentrations, following SSRI treatment, causes a down-regulation of neuronal firing through the cell body pre-synaptic 5-HT<sub>1A</sub> auto-receptor in the dorsal raphe. The dorsal raphe neurons project widely to other brain areas and firing down-regulation results in *reduced* serotonin production at the synaptic termini. Furthermore until the cell body 5-HT<sub>1A</sub> receptor desensitizes, a process that can take 2–3 weeks, serotonin production remains reduced.

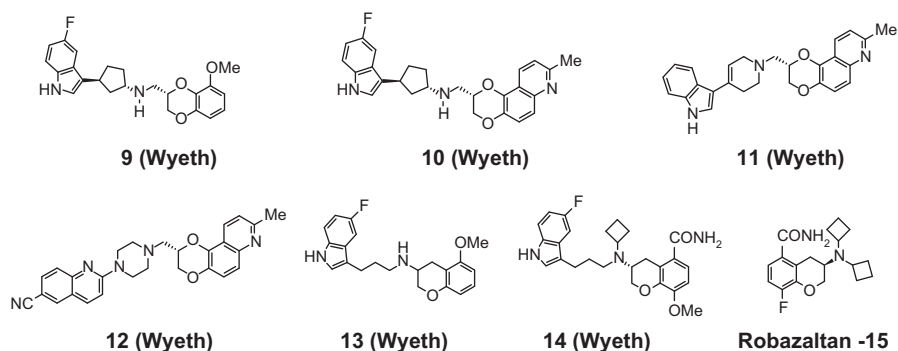


Knowing that 5-HT<sub>1A</sub> receptor activation was responsible for controlling serotonin production prompted Hjorth to antagonize the 5-HT<sub>1A</sub> receptor with penbutolol following treatment with citalopram.<sup>23</sup> This resulted in an overproduction of serotonin when compared to citalopram alone and stimulated the ongoing search for dual SRI/5-HT<sub>1A</sub> antagonists with the potential for rapid onset of antidepressant action. Support for the dual SRI/5-HT<sub>1A</sub> approach has been provided by clinical studies with antidepressants and pindolol and has supported a reduction in time to onset of action for the combined clinical treatments.<sup>24,25</sup>

The medicinal chemistry efforts adopted by several pharmaceutical companies to find combined serotonin reuptake inhibition and 5-HT<sub>1A</sub> antagonism in a single molecule started with patent filings in 1997. Initial efforts utilized pindolol (**5**) for 5-HT<sub>1A</sub> affinity and sought to incorporate a reuptake moiety, in place of the isopropylamine functionality of pindolol, resulting in **6** and analogues.<sup>26,27</sup> Following a pindolol replacement strategy researchers at the University of Navarra found that a series of aryl ketopiperazines, *e.g.* **7**, derived from benzyl alcohols, had 5-HT<sub>1A</sub> antagonist and serotonin reuptake inhibitor (SRI) activity, which translated into potent activity in an 8-OH-DPAT model of hypothermia.<sup>28</sup> Lundbeck chemists also reported on a series of indolylpiperazines, *e.g.* **8**, and demonstrated high affinity for both 5-HT<sub>1A</sub> and serotonin transporter (SERT) receptors.<sup>29,30</sup>

Active investigation by Wyeth has led to multiple patents directed towards SRI/5-HT<sub>1A</sub> antagonists. From 2004 onwards Wyeth have developed a series of benzopyrans (**9–11**) (Figure 8.2) and have identified and tackled many of the issues associated with the development of dual agents.<sup>31–33</sup>

In particular the difficulty associated with maintaining 5-HT<sub>1A</sub> antagonist activity was an issue for the Wyeth chemists and they developed strategies (*e.g.* selected halogenations) to confer antagonist function. Additionally problematic  $\alpha$ -1 adrenergic affinity (associated with postural hypotension) was noted with these platforms. The pyran series has culminated in **12**, which has balanced SERT ( $K_i$  13.8 nM) and 5-HT<sub>1A</sub> affinity ( $K_i$  3.7 nM) and has reported



**Figure 8.2** Robazaltan and serotonin reuptake inhibitor/5-HT<sub>1A</sub> antagonists.



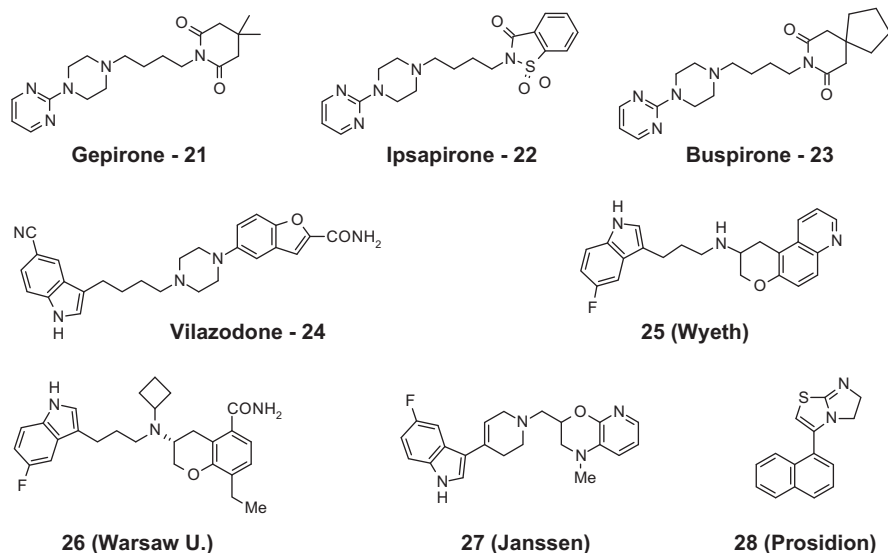
of SRI/5-HT<sub>1A</sub> platforms could be towards the development of molecules that contain overlapping pharmacophoric elements, as this would positively impact molecular weight and solubility.

### 8.2.2 Serotonin Reuptake Inhibition and 5-HT<sub>1A</sub> Agonism

A confounding feature in understanding the function of the 5-HT<sub>1A</sub> receptor is the differential functions of the pre-synaptic 5-HT<sub>1A</sub> auto-receptor and the post-synaptic 5-HT<sub>1A</sub> receptor. As mentioned in Section 8.2.1 the 5-HT<sub>1A</sub> auto-receptor is responsive to SRI-induced elevation of serotonin concentrations and undergoes down-regulation, manifested by a reduction in neuronal firing. The post-synaptic 5-HT<sub>1A</sub> receptor does not undergo down regulation in response to SSRIs and functions normally.<sup>43</sup> Furthermore the effect of 5-HT<sub>1A</sub> agonists on neuronal firing mirrors that of SSRI treatment. Thus gepirone (**21**) and ipsapirone (**22**) are 5-HT<sub>1A</sub> agonists that cause an acute reduction of neuronal firing. After 2 days pre-treatment with ipsapirone (**22**) (15 mg/kg/day), neuronal firing was reduced by 75%, but following 7 days pre-treatment firing was reduced by 15% and after 14 days firing rates were not different from controls.<sup>44,45</sup> Ipsapirone (**22**) has also undergone placebo-controlled clinical trials for depression and, while effective as an antidepressant, shares with SSRIs a delay in onset of action of 2–3 weeks.<sup>46</sup>

To further clarify the function of the post-synaptic 5-HT<sub>1A</sub> receptor a clinical trial was undertaken wherein pindolol and buspirone (**23**) were co-administered.<sup>47</sup> The rationale behind this experiment was to gauge the impact of a 5-HT<sub>1A</sub> pre-synaptic auto-receptor antagonist (pindolol – **5**) in the presence of a pre- and post-synaptic 5-HT<sub>1A</sub> agonist (buspirone – **23**). Pindolol (**5**) does not function as a post-synaptic 5-HT<sub>1A</sub> antagonist. The expectation was that in the absence of pre-synaptic auto-receptor agonism (*i.e.* pindolol **5**) blocking the effect of buspirone (**23**), what would remain would be a post-synaptic 5-HT<sub>1A</sub> agonist effect due to buspirone (**23**). This combination had a rapid antidepressant effect with remission observed within 7 days, underscoring the importance of the post-synaptic 5-HT<sub>1A</sub> receptor in depression treatment. The above observations support the incorporation of 5-HT<sub>1A</sub> agonist activity (preferably post-synaptic) into a therapeutic regime for depression and dual SRI/5-HT<sub>1A</sub> agonist combinations have been reported.

Thus Merck KGaA reported on a series of cyanoindolylbutyl piperazines and following an extensive SAR they identified vilazodone (**24**) (Figure 8.4), which was a potent 5-HT<sub>1A</sub> agonist (IC<sub>50</sub> = 0.3 nM, EC<sub>50</sub> (GTPγS binding) = 1.1 nM) and SERT inhibitor (IC<sub>50</sub> = 0.5 nM).<sup>48,49</sup> The functional 5-HT<sub>1A</sub> status of vilazodone has been contradicted by [<sup>35</sup>S]GTPγS binding studies in rat hippocampal membranes (a functional preparation where 5-HT<sub>1A</sub> receptors predominate), wherein vilazodone was shown to be a 5-HT<sub>1A</sub> receptor partial agonist with an EC<sub>50</sub> of 7.9 nM and an intrinsic activity of 0.61.<sup>50</sup> While there is ambiguity surrounding the 5-HT<sub>1A</sub> functional status of vilazodone, what is not in doubt is the clinical efficacy of this drug.<sup>51</sup> Vilazodone (**24**) has undergone a randomized, double-blind, placebo-controlled trial in 410 subjects with major



**Figure 8.4** 5-HT<sub>1A</sub> agonists, vilazodone and serotonin reuptake inhibitor/5-HT<sub>1A</sub> agonists.

depressive disorder (MDD) with doses from 10–40 mg/day over 2 weeks and was assessed for efficacy using the mean change from baseline to week 8 on the Montgomery–Asberg Depression Rating Scale (MADRS), HAM-D-17 and Hamilton Rating Scale for Anxiety. Response rates were determined at week 8 for the MADRS, HAM-D-17 and Clinical Global Impressions-Severity of Illness (CGI-S) and -Improvement (CGI-I) scales. Significant improvements in MADRS and HAM-D-17 scores were observed at week 1, the earliest time point measured. Response rates with vilazodone (**24**) were significantly higher than with placebo on the MADRS, HAM-D-17 and CGI-I scales. Treatment-emergent adverse events with vilazodone included diarrhoea, nausea and somnolence and most adverse events were mild or moderate. In summary vilazodone (**24**) was found to be effective for the treatment of MDD in adults, with symptomatic relief starting at 1 week, and was tolerated at a dose of 40 mg/day.<sup>52</sup> In January 2011 the FDA approved vilazodone (**24**) for the treatment of major depressive disorder and major depression.

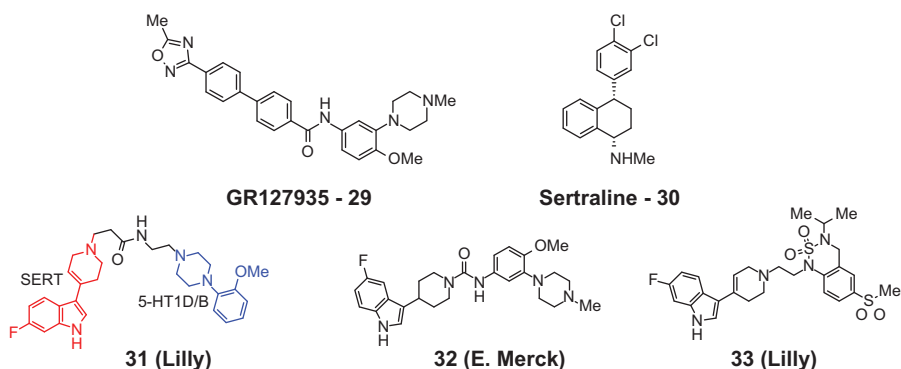
Despite the progress of vilazodone (**24**) few patent filings have been made that claim SRI/5-HT<sub>1A</sub> agonist activity. Wyeth have filed on a series of pyranoquinolinamines (*e.g.* **25**) as dual SRI/ 5-HT<sub>1A</sub> agonists, compounds that are derived from their more extensive SRI/5-HT<sub>1A</sub> antagonist effort<sup>53</sup> (see Section 8.2.1). There are also individual examples of SRI/5-HT<sub>1A</sub> agonists from within the University of Warsaw benzopyran SAR.<sup>41</sup> Interestingly in this latter SAR a minor change in structure from a methoxy to an ethyl group (**26**) converts the platform from antagonist to agonist functionality. Two additional patents of

note are due to Janssen and Prosidion. The former have developed a series of pyridooxazines (**27**) as antipsychotics with the primary activity being SERT inhibition, 5-HT<sub>1A</sub> agonism and D<sub>4</sub> antagonism. Prosidion are unique in developing a non-indole SRI/5-HT<sub>1A</sub> platform (**28**); however, they include norepinephrine reuptake inhibition in their platform profile and have patented for metabolic disorders, not depression.<sup>54,55</sup>

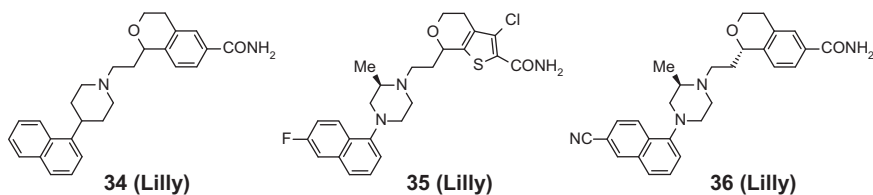
### 8.2.3 Serotonin Reuptake Inhibition and 5-HT<sub>1D</sub> Antagonism

In addition to being controlled by the pre-synaptic 5-HT<sub>1A</sub> auto-receptor, release of 5-HT is also controlled by the 5-HT<sub>1D</sub> receptor in man (and guinea pig), the 5-HT<sub>1B</sub> receptor is the species homologue in rat controlling release of 5-HT.<sup>56</sup> Both the 5-HT<sub>1D</sub> and the 5-HT<sub>1B</sub> receptors are located on the termini of serotonergic nerves. Additionally and analogously to the 5-HT<sub>1A</sub> receptor, blockade of the 5-HT<sub>1D</sub> auto-receptor in the presence of an SSRI causes a supra-maximal elevation of 5-HT production. Thus, in microdialysis studies in the guinea pig, administration of GR127935 (**29**) (Figure 8.5), a mixed 5-HT<sub>1B/D</sub> antagonist, at 5 mg/kg significantly increased extra-cellular levels of 5-HT to 135% of baseline values; additionally the SSRI sertraline (**30**) at 2 mg/kg increased 5-HT levels to 130% of baseline levels. The combination of sertraline and GR127935 (**29**) resulted in an increase of 5-HT levels to 230% of baseline values. This synergistic increase in 5-HT production suggested that a combined SRI/5-HT<sub>1D</sub> antagonist could be useful as a rapidly acting antidepressant.<sup>57</sup>

The first reports of SRI/5-HT<sub>1D</sub> antagonists appeared in patents in 1999.<sup>58,59</sup> The medicinal chemistry strategy that was adopted in the development of **31** and **32** paralleled the SRI/5-HT<sub>1A</sub> approach *i.e.* the combination of a SERT inhibitor (highlighted in red in structure **31**) with either a 5-HT<sub>1D</sub> or a 5-HT<sub>1B/D</sub> pharmacophore (highlighted in blue in structure **31**) joined *via* linking functionality. E. Merck have detailed the development of the SAR of **32** and



**Figure 8.5** GR127935, sertraline and serotonin reuptake inhibitor/5-HT<sub>1D</sub> antagonists.



**Figure 8.6** Serotonin reuptake inhibitor/5-HT<sub>1D</sub> antagonists.

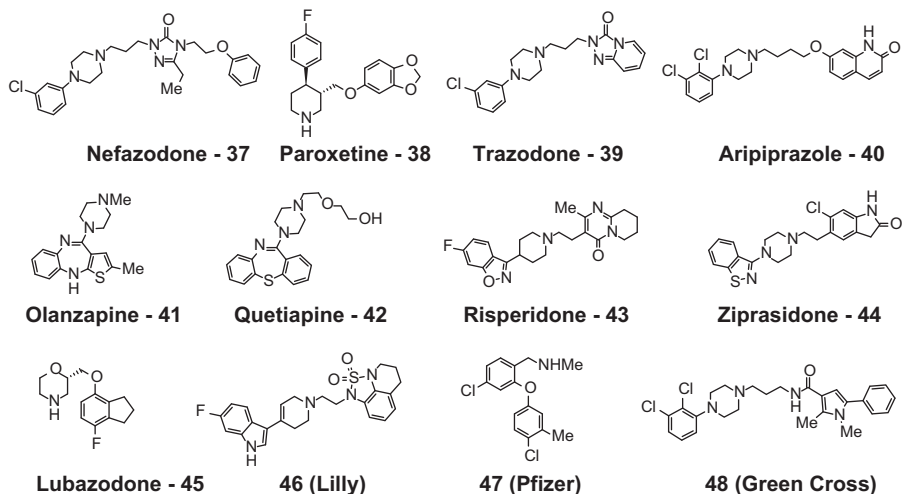
in doing so investigated a number of SERT pharmacophores. SERT activity was difficult to achieve with this platform (**32**, IC<sub>50</sub> SERT = 21 nM, IC<sub>50</sub> 5-HT<sub>1D</sub> = 233 nM) as a requirement for a centrally located basic nitrogen for SERT affinity was identified; however, this was detrimental to 5-HT<sub>1D/B</sub> affinity.<sup>60</sup>

Potent SERT affinity was observed with the benzothiadiazine 2,2-dioxide (**33**), which is reported to have SERT K<sub>i</sub> = 2.3 nM, K<sub>i</sub> versus 5-HT<sub>1D</sub> = 148 nM and significant affinity for 5-HT<sub>2A</sub>.<sup>61</sup> Microdialysis studies in the guinea pig showed that **33** was capable of producing a supra-maximal release of serotonin (800% increase) compared to fluoxetine (**4**) (350%) following acute dosing in guinea pigs.<sup>62</sup> While initial compounds in this series were mixed 5-HT<sub>1B/D</sub> antagonists, more recent SAR has attempted to develop selective SRI/5-HT<sub>1D</sub> agents **34–36** (Figure 8.6); thus, a series of benzopyran related molecules have been investigated culminating in the identification of cyanonaphthylpiperazine (**36**), which has K<sub>i</sub>s for SERT and 5-HT<sub>1D</sub> of 2.2 nM and 0.9 nM, respectively.<sup>63–65</sup>

Comparative microdialysis studies in the guinea pig showed that while fluoxetine (**4**) (20 mg/kg, po) gave a 200% increase in extra-cellular 5-HT concentration, compared to basal levels of 5-HT, **37** at 10 mg/kg, po, produced a 900% increase in 5-HT, reflecting the concomitant blockade of the serotonin transporter and the 5-HT<sub>1D</sub> receptor.<sup>65</sup>

### 8.2.4 Serotonin Reuptake Inhibition and 5-HT<sub>2A</sub> Antagonism

The development of SRI/5-HT<sub>2A</sub> antagonists stems from the clinical experience with nefazodone (**37**) (Figure 8.7). In a double-blind comparison between nefazodone (**37**) and paroxetine (**38**) in patients with moderate to severe depression there was no significant difference in clinical outcome.<sup>66</sup> In a second trial comparing the efficacy and effects on sexual function of nefazodone (**37**) and sertraline (**30**) there was no difference in antidepressant outcomes; however, sertraline (**30**) was reported to have a negative effect on sexual function whereas there was no dysfunction with nefazodone (**37**).<sup>67</sup> In a third study nefazodone (**37**) and fluoxetine (**4**) were compared for their effects on sleep architecture and quality of sleep in depressed patients.<sup>68</sup> Nefazodone (**37**) and fluoxetine (**4**) were found to be equally effective as antidepressants. In an assessment of sleep quality, fluoxetine increased the number of awakenings and did not alter sleep efficiency; however, nefazodone (**37**) increased sleep



**Figure 8.7** Atypical antipsychotics, paroxetine and serotonin reuptake inhibitor/5-HT<sub>2A</sub> antagonists.

efficiency and reduced the number of awakenings and the percentage of time awake. Both nefazodone (**37**) and a trazodone (**39**) (also an SRI/5-HT<sub>2A</sub> antagonist) have been evaluated in numerous clinical trials and found to be effective antidepressants.<sup>69–71</sup> Nefazodone (**37**) (SERT K<sub>i</sub>, 200 nM) and trazodone (**39**) (SERT K<sub>i</sub>, 160 nM) are relatively weak SERT inhibitors but both have high affinity for 5-HT<sub>2A</sub> (nefazodone (**37**) 5-HT<sub>2A</sub> K<sub>i</sub>, 5.8 nM and trazodone (**39**) 5-HT<sub>2A</sub> IC<sub>50</sub>, 17 nM).<sup>72,73</sup> Consistent with their 5-HT<sub>2A</sub> receptor affinity both nefazodone (**37**) and trazodone (**39**) showed improvements over placebo in HAMD sleep scores.<sup>74,75</sup>

Additionally, as the major atypical antipsychotics have found general applicability in depression augmentation (add on to SSRI) in the clinic (Table 8.1), it is appropriate to consider the binding profiles of these compounds. All the atypicals have a significant receptor affinity for the 5-HT<sub>2A</sub> receptor and may owe their efficacy in augmentation, in part, to this interaction.<sup>76,77</sup>

The observation of the effectiveness of nefazodone (**27**) and trazodone (**39**) as antidepressants, their ability to improve sleep quality and the clinical effectiveness of augmentation of SSRI therapy with antipsychotics has driven the search for combined SRI/5-HT<sub>2A</sub> antagonists. Thus, Yamanouchi developed an indanylmorpholine, lubazodone (**45**). Lubazodone (**45**) is an improved SERT inhibitor (SERT K<sub>i</sub>, 21 nM) compared to nefazodone (**27**) and trazodone (**39**) but had weaker affinity towards 5-HT<sub>2A</sub> (5-HT<sub>2A</sub> K<sub>i</sub>, 86 nM).<sup>78</sup> Additionally a benzothiadiazole dioxide has been appended to an indolyl tetrahydropyridine to obtain a dual SRI/5-HT<sub>2A</sub> antagonist. This resulted in the identification of **46**, which was a potent SERT and 5-HT<sub>2A</sub> antagonist (SERT K<sub>i</sub>, 2.3 nM; 5-HT<sub>2A</sub> K<sub>i</sub>, 0.81 nM).<sup>72</sup> More recently a QSAR study by Pfizer identified a phenoxybenzylamine platform (e.g. **47**) with high affinity for both



**Table 8.1** Receptor affinities of the atypical antipsychotics.<sup>77</sup>

		<i>5-HT<sub>1A</sub></i>	<i>5-HT<sub>2A</sub></i>	<i>5-HT<sub>2B</sub></i>	<i>5-HT<sub>2C</sub></i>	<i>5-HT<sub>6</sub></i>	<i>5-HT<sub>7</sub></i>	<i>D<sub>1</sub></i>	<i>D<sub>2</sub></i>	<i>D<sub>3</sub></i>	<i>D<sub>2</sub>: 5-HT<sub>2A</sub></i>
Aripiprazole ( <b>40</b> )	K <sub>i</sub> (nM)	5.6	17.5	0.36	22.4	574	10	387	0.95	4.5	0.05
Olanzapine ( <b>41</b> )	K <sub>i</sub> (nM)	2063	4.9	11.8	14.2	6	105	58	72	63	14.69
Quetiapine ( <b>42</b> )	K <sub>i</sub> (nM)	431	526	NA	1843	1864	308	712	567	483	1.08
Risperidone ( <b>43</b> )	K <sub>i</sub> (nM)	427	0.48	41.6	33.4	2241	6.6	60.6	4.9	12.2	10.21
Ziprasidone ( <b>44</b> )	K <sub>i</sub> (nM)	76	0.73	NA	13	61	6	30	4	17	5.48

SERT and 5-HT<sub>2A</sub> (SERT K<sub>i</sub>, 4.3 nM; 5-HT<sub>2A</sub> K<sub>i</sub>, 9 nM). The SAR study concluded that the ideal platform should have a low barrier to rotation around the diphenyl ether bond and that electron donating groups are preferred for greatest flexibility, which in turn allows access to the highest 5-HT<sub>2A</sub> affinity conformations.<sup>79</sup> Korean workers have hybridized an arylpiperazine with a pyrrole carboxamide to obtain **48**, an SRI/5-HT<sub>2A</sub> antagonist (SERT K<sub>i</sub>, 62 nM; 5-HT<sub>2A</sub> K<sub>i</sub>, 46 nM); **48** also had significant 5-HT<sub>2C</sub> affinity (5-HT<sub>2C</sub> K<sub>i</sub>, 21 nM).<sup>80</sup>

### 8.2.5 Serotonin Reuptake Inhibition and 5-HT<sub>3</sub> Antagonism

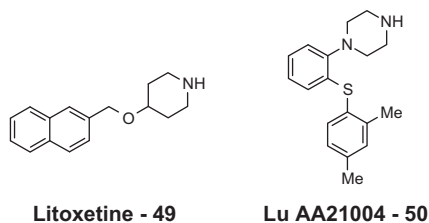
The increased availability of serotonin following SSRI treatment causes desirable (antidepressant) therapeutic effects and undesirable (nausea, sleep disturbances) side-effects.<sup>81</sup> Nausea following treatment with SSRIs is common: as many as 10–15% of patients being treated with fluoxetine (**4**) or paroxetine (**38**) report nausea as a side-effect.<sup>82</sup> The 5-HT receptor that induces nausea is primarily the 5-HT<sub>3</sub> receptor (both centrally and in the gastrointestinal tract).<sup>81</sup>

In support of the antidepressant potential of the combination SRI/5-HT<sub>3</sub> it has been demonstrated that the release of norepinephrine (NE) from the rat hippocampus is under the control of 5-HT acting through the 5-HT<sub>3</sub> receptor and that the inhibition of release of NE by 5-HT can be blocked by odansetron.<sup>83</sup> Accordingly dual SRI/5-HT<sub>3</sub> antagonists have been developed; however, this is a relatively unexplored pharmacological combination and has only been investigated sporadically. Thus litoxetine (**49**) (Figure 8.8) has high affinity for SERT (SERT K<sub>i</sub>, 7 nM) but more modest 5-HT<sub>3</sub> affinity (5-HT<sub>3</sub> K<sub>i</sub>, 315 nM).<sup>84–86</sup>

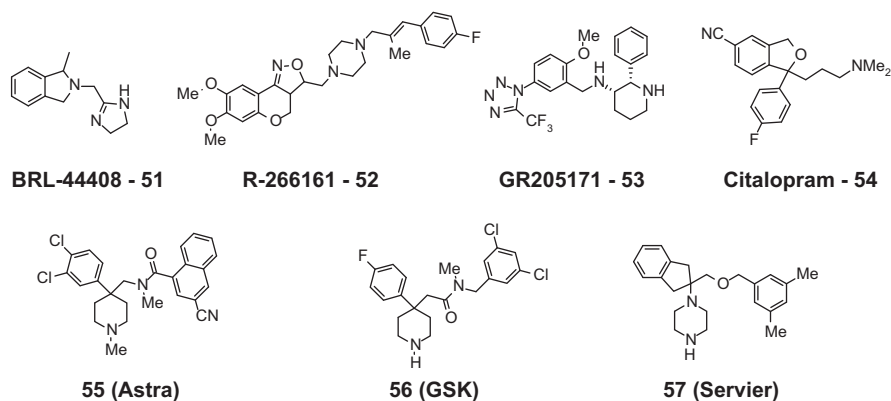
More recently a series of phenylsulfanylphenyl piperazines with SRI/5-HT<sub>3</sub> antagonist activity have been reported to be under development for the treatment of depression; thus, Lu AA21004 (**50**) has balanced SERT and 5-HT<sub>3</sub> affinity (SERT K<sub>i</sub>, 1.6 nM; 5-HT<sub>3</sub> K<sub>i</sub>, 3.7 nM). Lu AA21004 (**50**) also has affinity for the 5-HT<sub>1A</sub> receptor (5-HT<sub>1A</sub> K<sub>i</sub>, 15 nM) and is a 5-HT<sub>1A</sub> partial agonist.<sup>87,88</sup>

### 8.2.6 Serotonin Reuptake Inhibition and Non-serotonergic Receptor Interaction

The finding that the selective  $\alpha_{2A}$  adrenergic receptor antagonist BRL-44408 (**51**) when co-administered with fluoxetine (**4**) elevated extra-cellular levels of



**Figure 8.8** Litoxetine and Lu AA21004.



**Figure 8.9** BRL-44408 and serotonin reuptake inhibitor/ $\alpha_{2A}$  adrenergic receptor antagonists.

both 5-HT and norepinephrine has prompted the search for mixed SRI/ $\alpha_{2A}$  adrenergic receptor antagonists.<sup>89</sup> The rationale for the increase in NE levels is through pre-synaptic auto-receptor  $\alpha_{2A}$  antagonism. Blockade of the  $\alpha_{2A}$  receptor allows for continued NE production, which would otherwise be subject to feedback control. The enhanced NE production then agonizes the  $\alpha_1$  heteroreceptor on serotonergic neurons, thereby stimulating 5-HT production.<sup>90,91</sup> In searching for SRI/ $\alpha_{2A}$  adrenergic receptor antagonists Johnson & Johnson have investigated a series of tricyclic isoxazolines, which potently antagonize the  $\alpha_2$  receptors and inhibit 5-HT uptake. Thus R-266161 (**52**) (Figure 8.9) inhibits  $\alpha_{2A}$ ,  $\alpha_{2B}$ ,  $\alpha_{2C}$  with  $K_i$ s of 3.7, 1.5 and 0.27 nM, respectively, as well as inhibiting SERT ( $K_i$ , 1.7 nM).

Other combinations that have been investigated include SRI/ $NK_1$  antagonists, which may have enhanced antidepressant effects. Thus the  $NK_1$  antagonist GR205171(**53**) when co-administered with either citalopram (**54**) or fluoxetine (**4**) causes a 245–343% increase in basal 5-HT production and the antidepressant effect of citalopram was enhanced by GR205171 (**53**) in the forced-swim test (FST).<sup>92</sup>

Dual SRI/ $NK_1$  antagonists have been developed, typified by the piperidines developed by Astra (**55**) and GSK (**56**), but few biological data have been published on these series.<sup>93,94</sup> Servier have developed a series of spirocyclic indanes (e.g. **57**) that have balanced SERT and  $NK_1$  affinity with  $pK_i$ s vs. SERT of 7.27 and  $NK_1$  7.74, respectively.<sup>95</sup>

### 8.2.7 Serotonin and Norepinephrine Reuptake Inhibition

While the effectiveness of SSRIs is comparable to tricyclic antidepressant studies, in geriatric populations with severe depression (high on the Hamilton depression rating scale) it has been demonstrated that tricyclic antidepressants are more effective than SSRIs.<sup>96,97</sup> As the tricyclic antidepressants are dual SERT/norepinephrine transporter (NET) inhibitors (imipramine (**58**) SERT

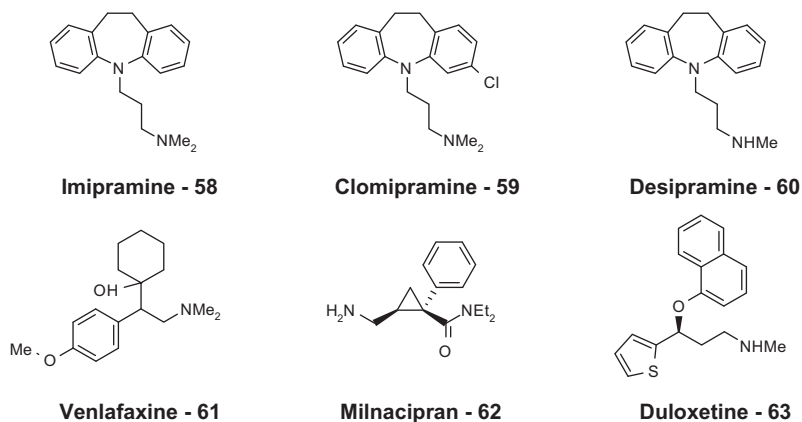
$K_i$ , 14 nM; NET  $K_i$ , 41 nM; clomipramine (**59**) SERT  $K_i$ , 18 nM; NET  $K_i$ , 60 nM), though encumbered by unwanted pharmacology, a cleaner tricyclic antidepressant reliant on SERT and NET inhibition alone could be a superior antidepressant.<sup>98</sup> This hypothesis has been tested and it has been found that co-administration of fluoxetine (**4**) and desipramine (**60**) is more likely to result in remission as assessed on the MADRS than either fluoxetine (**4**) or desipramine (**60**) alone. With the combination treatment 53.8% remission was achieved, while with fluoxetine (**4**) or desipramine (**60**) 7.1% and 0%, respectively, achieved remission. However, there was not a statistically meaningfully faster rate of onset of action with the combination of fluoxetine (**4**) and desipramine (**60**).<sup>99</sup>

Dual SERT/NET inhibitors are marketed for depression, with venlafaxine (**61**) (Figure 8.10), which was first prepared in 1984, launched in 1993.<sup>100–103</sup> This was followed by milnacipran (**62**), launched in 1996, and duloxetine (**63**), which was launched in 2004.<sup>104–111</sup> A feature of the antidepressant profile of venlafaxine (**61**) is its rapid onset of action compared to SSRIs.<sup>112</sup> The potential for superior efficacy and earlier onset has prompted interest in dual inhibitors with 27 platforms being patented since 2000. Clinical experience with dual uptake inhibitors has revealed additional therapeutic utility as dual SERT/NET inhibitors are now being developed for neuropathic pain and stress urinary incontinence (SUI).<sup>113</sup>

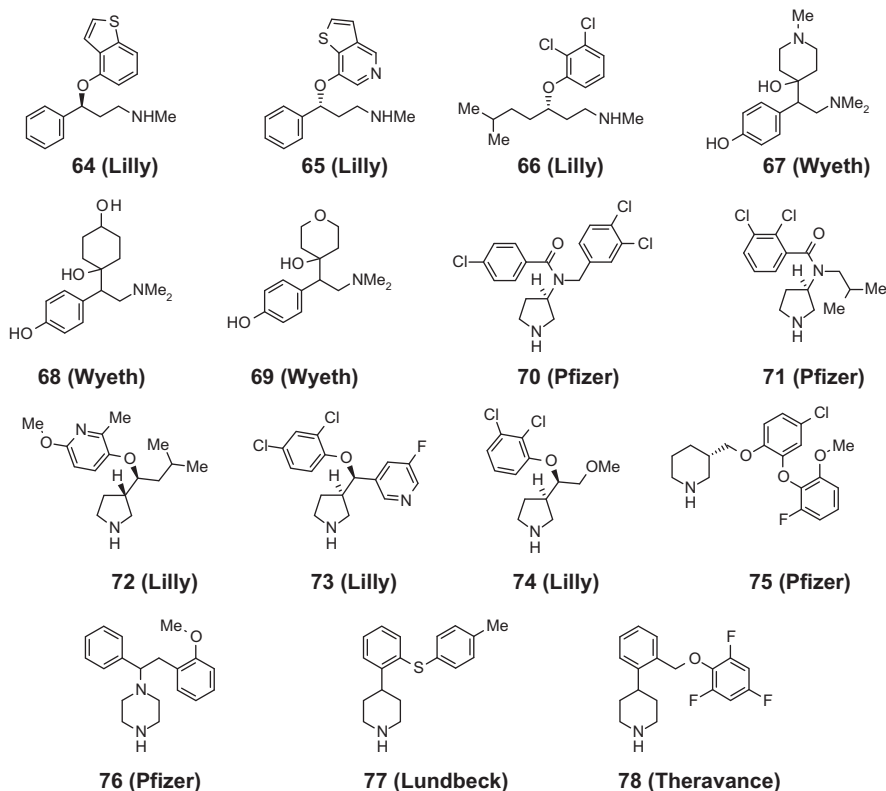
The newer dual SERT/NET inhibitors analogues that have been prepared fall broadly into four categories: duloxetine analogues, venlafaxine analogues, 3-substituted pyrrolidines and arylated piperidines. A number of miscellaneous structures have been developed.<sup>114</sup>

A series of *N*-methylpropanamines (**64–66**) (Figure 8.11) related in structure to duloxetine have been investigated and heterocyclic alternatives to naphthalene were found to be potent dual SERT/NET inhibitors.<sup>115–117</sup>

Venlafaxine (**61**) has been shown to be extensively metabolized and the *O*-desmethyl analogue (**67**) has been identified as a major metabolite in man.<sup>118</sup>



**Figure 8.10** Imipramine, clomipramine, desipramine and dual serotonin/nor-epinephrine reuptake inhibitors.



**Figure 8.11** Dual serotonin/norepinephrine reuptake inhibitors.

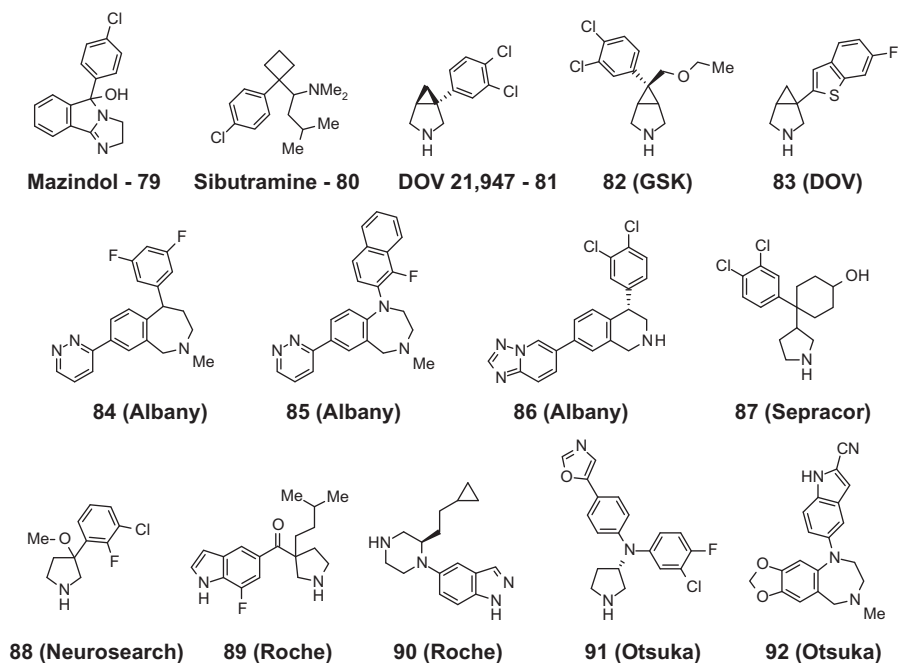
The *O*-desmethyl metabolite (**67**) exhibits an antidepressant profile and inhibits serotonergic and noradrenergic receptor firing similar to venlafaxine (**61**), though with reduced potency. Accordingly close analogues of venlafaxine that are phenol derivatives (**68**, **69**) have been prepared.<sup>119–121</sup>

Newer platforms that have emerged are based on 3-substituted pyrrolidines and for acceptable dual activity these platforms need a lipophilic fragment, which can be either an aromatic ring (**70**) or an alkyl chain (**71**).<sup>122–126</sup> Additionally a functional group bearing an H-bond acceptor (amide carbonyl, ether, pyridyl nitrogen) moiety is usually present. Pfizer have developed the isobutyl benzamide (**72**) after reducing p-glycoprotein interaction; **72** has  $K_{is}$  vs. SERT and NET of 6 and 21 nM, respectively, as well as brain:plasma = 0.45.<sup>123</sup> Alternatives to the heterocyclic ring have also been identified (**73**, **74**) and retained dual activity. The primary clinical targets for these dual inhibitors are either SUI or pain, not depression. Arylated-piperidines (**75**) and -piperazines (**76**) have been developed by Pfizer, Lundbeck (**77**) and Theravance (**78**).<sup>127–130</sup> Compound **76** is a balanced SERT ( $K_i$ , 2 nM) and NET ( $K_i$ , 12 nM) inhibitor with improved metabolic stability and reduced h-ERG ( $IC_{50}$ , 22.9  $\mu$ M) liability.<sup>128</sup>

## 8.2.8 Serotonin, Norepinephrine and Dopamine Reuptake Inhibition

Aberrant dopaminergic regulation and low CNS dopamine levels have been linked to depression and the severity of depressive episodes.<sup>131,132</sup> In particular the study of bipolar depressed patients has revealed linkages to CNS dopamine concentration. Using cerebrospinal fluid (CSF) levels of the principal metabolite of dopamine, homovanillic acid, as a surrogate marker for dopamine, correlation of high dopamine levels has been made with the manic phase of bipolar disorder and low dopamine levels to the depressive phase.<sup>133,134</sup> The bipolar depression correlate has implicated low dopamine in anhedonic states and as anhedonia is not addressed by SERT/NET inhibitors the introduction of dopamine reuptake inhibition to dual inhibitors was suggested and the identification of triple reuptake inhibitors (TRUIs) initiated.<sup>135,136</sup>

An early TRUI was mazindol (**79**) (Figure 8.12), which found use for the treatment of obesity but has been discontinued.<sup>137</sup> Mazindol exists as a tautomer between the uncyclized (4-chlorophenyl)-[2-(4,5-dihydro-1H-imidazol-2-yl)phenyl] methanone and mazindol (5-(4-chlorophenyl)-2,3-dihydroimidazo[2,1-a]isoindol-5-ol) (**79**). Mazindol is the active tautomer and has modest affinity for the transporters with IC<sub>50</sub>s *versus* SERT, NET and dopamine transporter (DAT) of 50, 18 and 45 nM, respectively.<sup>138</sup>



**Figure 8.12** DOV 21,947 and triple serotonin/norepinephrine/dopamine reuptake inhibitors.

No TRUIs are marketed for depression at this time; however, the pharmacology of sibutramine (**80**), a largely discontinued anti-obesity drug, is noteworthy.<sup>139</sup> Sibutramine (**80**) was initially reported to be a dual SERT/NET uptake inhibitor but further evaluation revealed that sibutramine induced potential-dependent exocytotic release of dopamine, which was not carrier mediated.<sup>140,141</sup> Studies with sibutramine have demonstrated that a pre-existing cardiovascular condition can be revealed and non-fatal myocardial infarction or stroke may result following long-term administration (5 weeks) and this may have implications for the development of newer TRUIs.<sup>142</sup>

The patenting of 3,4-dichlorophenyl-3-azabicyclo[3.1.0]hexane (**81**) by DOV Pharmaceuticals for depression was a seminal filing in the development of TRUIs as it identified two key pharmacophoric requirements: the 3,4-dichlorophenyl ring and a basic saturated nitrogen heterocycle.<sup>143</sup> These features have been heavily exploited and since 2005 over 50 patents having been filed claiming TRUIs for the treatment of depression that have used either the 3,4-dichlorophenyl ring or a bio-isosteric equivalent thereof (*e.g.* **82**, **83**).<sup>144,145</sup>

GlaxoSmithKline have filed extensively in the TRUI area and have developed several series relating to 3,4-dichlorophenyl-azabicyclo[4.1.0]heptanes and -azabicyclo[3.1.0]hexanes. Using pharmacophore models GlaxoSmithKline developed **82**, which is a balanced reuptake inhibitor with  $pK_i$  versus SERT, NET and DAT of 9.8, 9.3 and 8.7, respectively.<sup>146–150</sup>

The discovery of DOV 21,947 (**81**), which inhibits SERT, NET and DAT uptake with  $IC_{50}$ s of 12, 23 and 96 nM, respectively, prompted the preparation of analogues where the 3,4-chlorophenyl group has been replaced by substituted naphthyl ring systems or benzo-fused heterocycles.<sup>151–153</sup> Triple reuptake inhibition was retained with these modifications, thus **83** inhibits SERT, NET and DAT uptake, respectively, at 100%, 98% and 99% at 1  $\mu$ M.<sup>154</sup> The racemate of DOV 21,947 (**81**) (DOV 216,303) was under development for depression but has been terminated and DOV have reverted to developing DOV 21,947 (**81**).<sup>155</sup>

Albany Molecular chemists have developed a benzazepine series of TRUIs (**84**, **85**), which have evolved through a benzodiazepine platform to a tetrahydroisoquinoline platform.<sup>156–160</sup> The novel aspect of the three platforms is the appended heterocycle, which is a feature distinct to these platforms, the SAR having culminated with an appended triazolopyridine (**86**) moiety.<sup>161</sup>

Sepracor have also sought TRUIs by substituting a cyclohexanol ring in the 4-position with a geminal 3,4-dichlorophenyl ring and a pyrrolidin-3-yl group. They achieved the necessary pharmacophoric requirements in a constrained manner to give a rigid TRUI (**87**), which has  $pK_i > 8$  at all three transporters.<sup>162–165</sup>

A pyrrolidine series of TRUIs has also been disclosed by Neurosearch, thus **88** was evaluated in microdialysis studies and increased baseline levels of 5-HT, NE and DA by 327%, 660% and 459%, respectively, in rat prefrontal cortex.<sup>166</sup>

Roche have also pursued TRUIs, their most recent platforms being indolyl pyrrolidinyl ketones (**89**) and indazolyl piperazines (**90**).<sup>167,168</sup> The indolyl



pyrrolidinyl ketones have been reported to be under development for pain and are instructive as they highlight a difficulty in the development of poly-pharmacological drugs.

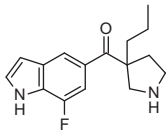
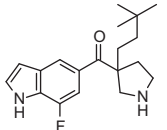
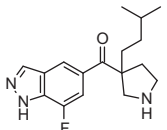
To facilitate translation to the clinic, equivalent receptor occupancies at SERT, NET and DAT would allow for facile dose selection following pharmacokinetic and pharmacodynamic modelling. The difficulty inherent in selecting compounds with equivalent occupancies is highlighted by the variation in DAT occupancy achieved following comparable dosing ( $3.9 \pm 0.1$  mg/kg) of three indolyl pyrrolidinyl ketone analogues that all achieve 80% occupancy at SERT (Table 8.2).<sup>167</sup>

Two distinct platforms have been developed by Otsuka Pharmaceutical, the first a series of diarylamino pyrrolidines (*e.g.* **91**), which produced balanced and potent SERT and NET inhibition and occasional DAT. Accordingly **91** is reported to inhibit SERT, NET and DAT uptake with  $IC_{50} = 1.2, 0.7, 4.8$  nM, respectively.<sup>169</sup>

The second Otsuka Pharmaceutical series, a benzodiazepine platform, has explored SAR space, which compares with an early Albany Molecular platform and has been optimized to produce potent and balanced triple reuptake inhibition with **92** having  $IC_{50}$ s *versus* SERT, NET and DAT of 5.6, 5.7 and 7.1 nM, respectively.<sup>170</sup>

An indolyindane platform (*e.g.* **93**) has been reported by Lundbeck primarily for obesity, and a tetrahydroisoquinoline series has been reported by Panacea Biotech; thus, **94** inhibits SERT, NET and DAT uptake, respectively,

**Table 8.2** Comparative SERT and DAT occupancy of ketopyrrolidine TRUIs.<sup>167</sup>

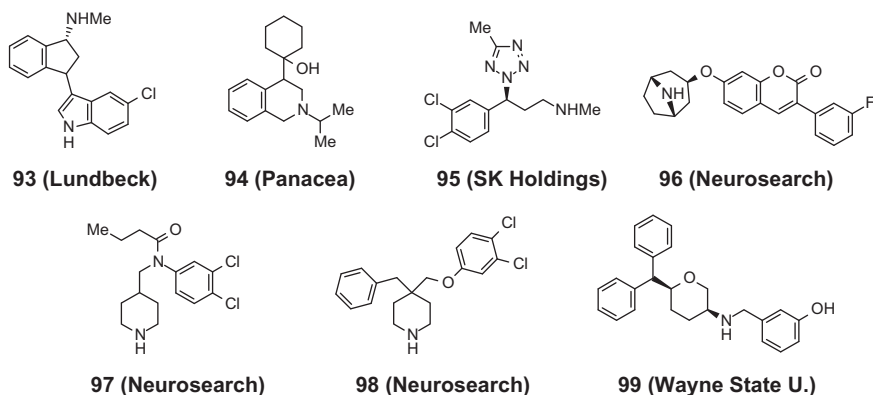
Structure	SERT $pK_i$	DAT $pK_i$	Dose giving 80% SERT occupancy mg/kg ( <i>ip</i> )	SERT:DAT occupancy ratio at 80% SERT occupancy
	8.8	7.8	4.0	80:31
	9.3	9.2	3.9	80:35
	8.6	8.2	3.8	80:67

at 49%, 36% and 48% at 10 nM.<sup>171,172</sup> SK Holdings have reverted to propa-namines in their quest for a TRUI for depression and found that the combination of 3,4-dichlorophenyl and tetrazole groups confers DAT inhibition in a platform usually associated with SERT and NET activity. Compound **95** inhibits SERT, NET and DAT uptake respectively by 43%, 84% and 62% at 100 nM.<sup>173</sup>

Through many iterations Neurosearch have worked to develop azabicyclo[3.2.1]octanes as TRUIs. Maintaining an azabicycloamine throughout, the initial compounds of this SAR were disubstituted phenyl rings, but the series has evolved through heterobicyclic aromatics and culminated in the chromene platform as exemplified by compound **96**, which has IC<sub>50</sub>s versus SERT, NET and DAT of 5.8, 26 and 28 nM, respectively.<sup>174–176</sup> Two series of piperidines have been investigated by Neurosearch, a piperidine amide series from which **97** was developed which has SERT, NET and DAT IC<sub>50</sub>s of 6.6, 7.2 and 86 nM, respectively, and a 4,4-disubstituted benzyl piperidine (**98**), which has an optimized 3,4-dichlorophenoxy ring that provides high affinity for all three transporters with SERT, NET and DAT IC<sub>50</sub>s of 0.26, 4.8 and 6 nM, respectively.<sup>177,178</sup> Wayne State University have investigated a series of pyrans and found that compound **99** has balanced triple reuptake inhibition with DAT, SERT and NET K<sub>i</sub>s of 31.3, 40 and 38.5 nM, respectively. Compound **99** was active in the FST at 10 mg/kg ip.<sup>179</sup>

### 8.2.8.1 Norepinephrine and Dopamine Reuptake Inhibition

Efforts to find dual NET/DAT transport inhibitors have not been as pronounced as the search for SNRIs or TRUIs, despite the clinical experience in the treatment of depression with nomifensine (**100**) (Figure 8.13) and bupropion (**101**).<sup>180,181</sup> Nomifensine (**100**) has modest affinity for the NE (NET K<sub>i</sub>, 29 nM) and dopamine (DAT K<sub>i</sub>, 53 nM) transporters and interacts weakly with



**Figure 8.13** Triple serotonin/norepinephrine/dopamine reuptake inhibitors.

the 5-HT transporter (SERT  $K_i$ , 4,872 nM).<sup>182</sup> Nomifensine (**100**) was withdrawn from the market following occurrences of haemolytic anaemia and kidney and liver toxicity. The toxicity of nomifensine (**100**) has been attributed to the formation of reactive metabolites, which in turn led to toxic glutathione conjugates.<sup>183</sup> Bupropion (**101**) has a low affinity for DAT and PET studies have revealed a low occupancy of the DAT transporter under steady-state oral dosing, with an average occupancy of 26% over a 24-h period.<sup>184</sup> Evidence for the inhibition of NET by bupropion (**101**) is weak; rather an NE releasing effect from the locus coeruleus has been proposed to account for its clinical efficacy.<sup>185</sup>

Seeking new treatments for depression, Organon chemists have explored an 8-azabicyclo[3.2.1]octane platform, which is reported to give representatives with SERT affinity and also dual NET/DAT active analogues. Compound **102** has  $pEC_{50} > 7$  at both norepinephrine and dopamine transporters.<sup>186</sup>

Neurosearch have reported three platforms that are dual NET/DAT inhibitors, the first a diazobicyclo[3.3.1]nonane (**103**), which, unusually for a reuptake inhibitor, has a carbamate linker to a substituted naphthalene ring and is reported to have NET and DAT  $IC_{50}$ s of 24 nM and 30 nM, respectively.<sup>187</sup> The other two platforms from Neurosearch are structurally related piperidine amides. The early series had a benzimidazolyl moiety and compound **104** from this platform had balanced NET and DAT inhibition (NET  $IC_{50}$ , 20 nM; DAT  $IC_{50}$ , 15 nM). The later series dispensed with the benzimidazoleethyl functionality and this resulted in improved transporter affinity with reported NET and DAT  $IC_{50}$ s of 2.6 nM and 3.1 nM, respectively, for **105**.<sup>188–190</sup>

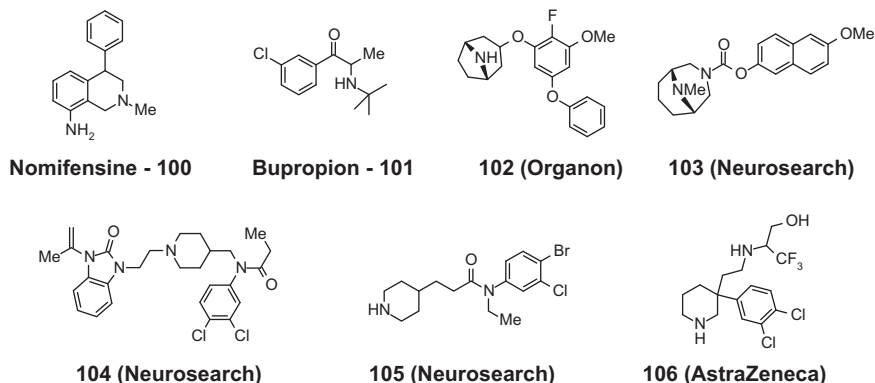
With the aim of finding a compound with the profile of nomifensine (**100**) without the toxicological liabilities, AstraZeneca have undertaken an extensive SAR around a 3,3-disubstituted piperidine platform resulting in nanomolar affinity NET and DAT inhibitors. Compound **106** has a hydroxyethylamino side chain with a beta trifluoromethyl group, presumably present to attenuate the amine basicity, the resulting combination of which gives NET and DAT  $K_i$ s of 1.7 nM and 1.3 nM, respectively.<sup>191</sup>

### 8.2.8.2 Norepinephrine Reuptake Inhibition

The development of dual and triple reuptake inhibitors has not diminished interest in finding new NRIs. This may be due to their clinical effectiveness in the treatment of attention deficit hyperactivity disorder (ADHD) and perimenopausal disorders of thermoregulation as well as depression therapy.<sup>192,193</sup>

The history of treatment with NRIs largely involves the use and profile of desipramine (**60**) (Figure 8.14). Desipramine (**60**) is a selective NET inhibitor ( $K_i$ , 3.8 nM), which is effective in depression and has a rapid speed of onset of antidepressant action.<sup>182,194</sup> As a tricyclic antidepressant, desipramine (**60**) also possesses affinity for histamine, muscarinic and alpha adrenergic receptors.<sup>195</sup>

The first NRI that has found use in the treatment of depression is reboxetine (**107**), which has been reported to be effective in multiple clinical trials, but this has been challenged following a meta-analysis of these clinical trials.<sup>196,197</sup>



**Figure 8.14** Nomifensine, bupropion and dual norepinephrine/dopamine reuptake inhibitors.

Accordingly reboxetine (**107**) is available in Europe for the treatment of depression but not in the United States.

Atomoxetine (**108**) is an NRI that has been used in the treatment of attention deficit hyperactivity disorder; an analogue of the SERT inhibitor fluoxetine (**4**) it was launched in 2003.<sup>198</sup>

Seeking NRIs for the treatment of pain, Gruenenthal chemists have identified a series of non-basic pyridopyrimidine amides, structurally unrelated to other NERIs. Compound **109** provides 100% inhibition of NET at 10  $\mu\text{M}$ .<sup>199</sup> A series of dihydroquinolinones have been developed as selective NERIs; thus, **110** has a NET  $K_i$  of 6 nM and SERT and DAT  $K_i$ s > 100 nM.<sup>200</sup> A series structurally related to the dihydroquinolinones are the benzothiadiazine dioxides, which have been patented by Wyeth for hot flushes. This SAR has led to the benzothiadiazolo-dioxide **111**, which has high affinity for NET ( $\text{IC}_{50}$ , 1 nM) and excellent selectivity over SERT (48% inhibition at 6  $\mu\text{M}$ ). Compound **111** has demonstrated *in vivo* activity in models of hot flushes and neuropathic pain.<sup>201,202</sup>

Hydroxyalkylamines (*e.g.* **112**, **113**) have been utilized by Wyeth in a second series that has also been investigated for hot flushes. Compound **113** has good affinity for NET ( $K_i$ , 4.1 nM), selectivity over SERT ( $K_i$ , 5,293 nM) and is active *in vivo* in a skin-temperature model of hot flushes, achieving >2.5  $^{\circ}\text{C}$  drop in mean skin temperature with an 8.5 h duration of action.<sup>203,204</sup>

Development of the morpholine platform has been undertaken and the pharmacophoric requirements exemplified by reboxetine (**107**) (basic nitrogen, phenyl ring, 2-substituted aryl ether) have been modified with the replacement of the aryl ether linkage (*e.g.* **114**, **115**) with retention of NET activity and selectivity over SERT.<sup>205,206</sup> In addition tertiary alcohol substituted carbon linked morpholines have been developed (*e.g.* **116**).<sup>207,208</sup> Pfizer have also re-examined the reboxetine platform and identified selective NET inhibitors; thus, compound (**117**) has a NET  $K_i$  of 1.91 nM (SERT  $K_i$ , 313.6 nM).<sup>209</sup>

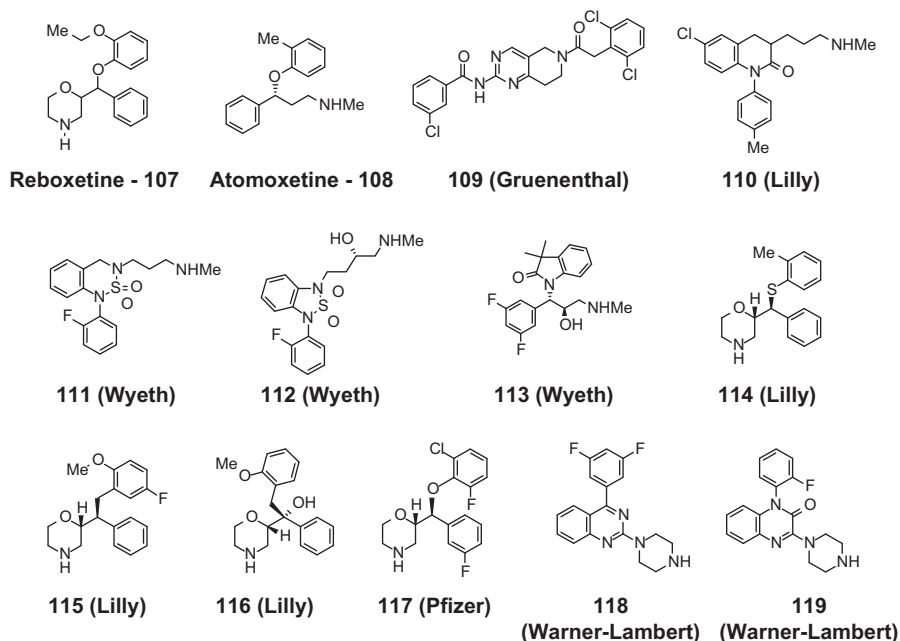
Two piperazine platforms have been developed by Warner-Lambert; the first, an aryl quinazoline (**118**), has a NET  $K_i$  of 3.7 nM and a SERT  $K_i$  of 107 nM, while the later platform, an aryl quinoxalinone (**119**), is reported to have NET  $K_i$  of 11 nM.<sup>210,211</sup>

### 8.3 Pre- and Post-synaptic 5-HT Targets

The principal receptors responding to the 5-HT released as a result of SSRI treatment have also been targeted in the search for new antidepressants. Of the multiple pre- and post-synaptic receptors that have been addressed, the receptors that have received the most attention are 5-HT<sub>1A</sub> and 5-HT<sub>2C</sub>. The 5-HT<sub>2A</sub> and 5-HT<sub>7</sub> receptors have been strongly implicated in depressive syndromes, notably sleep and circadian rhythm disturbances; however, no single agent has been advanced for depression.

#### 8.3.1 5-HT<sub>1A</sub> Agonism

As noted in Section 8.2.2, 5-HT<sub>1A</sub> agonists have been investigated for the treatment of depression. The exemplar 5-HT<sub>1A</sub> agonist/partial agonists are buspirone (**23**), gepirone (**21**) and tandospirone (**120**) (Figure 8.15), all of which derive from the aminopyrimidine platform. Buspirone (**23**), a partial 5-HT<sub>1A</sub> agonist, was launched in 1986 and is now generically available for the treatment



**Figure 8.15** Reboxetine, atomoxetine and selective norepinephrine reuptake inhibitors.

of generalized anxiety disorder (GAD).<sup>212</sup> In an antidepressant trial, a group of SSRI non-respondents (to fluoxetine (**4**), paroxetine (**38**), citalopram (**54**) or clomipramine (**59**)) were subsequently given buspirone (**23**) with concomitant co-administration of the “failed” antidepressant. After 5 weeks’ treatment with buspirone (**23**) 59% showed complete or partial remission of their depression symptoms.<sup>213</sup> Gepirone (**21**) has also been evaluated in depression trials and after 8 weeks 79% of patients responded, which was a response rate greater than that seen with desipramine (**60**). Additionally response rates with gepirone (**21**) were distinguishable from placebo in two weeks.<sup>214</sup> The azapirones as a class have a short half-life, which necessitates multiple dosings per day. This and their effectiveness in depression trials has prompted a renewed search for 5-HT<sub>1A</sub> agonists.<sup>215,216</sup>

Tandospirone (**120**) (5-HT<sub>1A</sub> K<sub>i</sub>, 27 nM), a partial 5-HT<sub>1A</sub> agonist, is marketed in China and Japan for GAD and depression.<sup>217,218</sup> Additionally the behavioural and psychological symptoms associated with dementia are lessened with tandospirone (**120**); thus, symptoms such as anxiety, depression, agitation and aggression were reduced significantly in an open labelled trial.<sup>219</sup>

Following the development of the piperazinopyrimidines, alternative 5-HT<sub>1A</sub> agonist platforms were sought. One of these, flesinoxan (**121**) (5-HT<sub>1A</sub> K<sub>i</sub>, 1.7 nM), was investigated for the treatment of depression but was not developed.<sup>220,221</sup> Flesinoxan (**121**) differs from the pyrimidinopiperazines in two respects: firstly the lipophilic imide is replaced by a benzamide and secondly the pyrimidine ring is modified to a benzodioxine ring. Flesinoxan (**121**) was also reported to enhance cognition in the over-75s, demonstrating beneficial effects on word recall, word recognition and reaction time.<sup>222</sup> 5-HT<sub>1A</sub> agonists have also been patented for neurodegenerative diseases and ischaemia; thus, Abbott developed a series of triazoles and achieved very high affinity with compound **122** at the 5-HT<sub>1A</sub> receptor (K<sub>i</sub> = 0.2 nM).<sup>223</sup> Additionally Abbott developed a second series of triazoles where the trifluoromethylpiperidine was replaced with an isoquinolylpiperazine.<sup>224</sup>

American Home Products investigated a series of adamantyl amides linked to a pyrimidinopiperazine (*cf.* buspirone (**23**)); however, their platform achieved novelty due to lipophilic substituents (*e.g.* phenyl, cyclohexyl) on the linking chain. Compound **123** had high affinity for 5-HT<sub>1A</sub> (K<sub>i</sub>, 0.43 nM) and was a full agonist (EC<sub>50</sub>, 2 nM).<sup>225</sup> Cepa Schwarz Pharma and Dainippon Sumitomo Pharma investigated structurally related chromans (*e.g.* **124**) and tetralins (*e.g.* **125**), which are characterized by having a secondary amine in place of a piperazine or piperidine.<sup>226,227</sup> The chroman series was developed for ischaemia and stroke and **124** is reported to have 5-HT<sub>1A</sub> K<sub>i</sub> of 1.23 nM and an EC<sub>50</sub> of 16.3 nM.<sup>226</sup>

Two structurally related platforms, one an isoquinolinone, the other a thienopyrimidinone, were patented by Knoll. The former (**126**) had high affinity for 5-HT<sub>1A</sub> (K<sub>i</sub>, 0.6 nM) and both platforms were developed for neurodegeneration and ischaemia.<sup>228,229</sup> Pfizer have developed a series of structurally distinct pyrimidinopiperidines: **127** has a modest K<sub>i</sub> at the 5-HT<sub>1A</sub> receptor (63 nM) and a 5-HT<sub>1A</sub> EC<sub>50</sub> of 40 nM. The series was optimized towards brain penetration and compound **127** had a brain:plasma ratio of 1.27.<sup>230</sup>

A series of fluoropiperidines was investigated by Pierre Fabre for their effects in FST and it was concluded that their efficacy in the FST was correlated with 5-HT<sub>1A</sub> agonist potency. The most efficacious compound was F-13714 (**128**) (5-HT<sub>1A</sub> pK<sub>i</sub>, 10.23), which was a full agonist.<sup>231</sup> This series was developed from the methylaminopyridine series and culminated in F-15599 (**129**) (5-HT<sub>1A</sub> pK<sub>i</sub>, 9.07).<sup>232</sup> F-15599 has also been shown to demonstrate preferential post-synaptic 5-HT<sub>1A</sub> receptor activation and has been evaluated in the FST and has an ED<sub>50</sub> of 0.12 mg/kg po and is also reported to be active in models of cognition.<sup>232–234</sup>

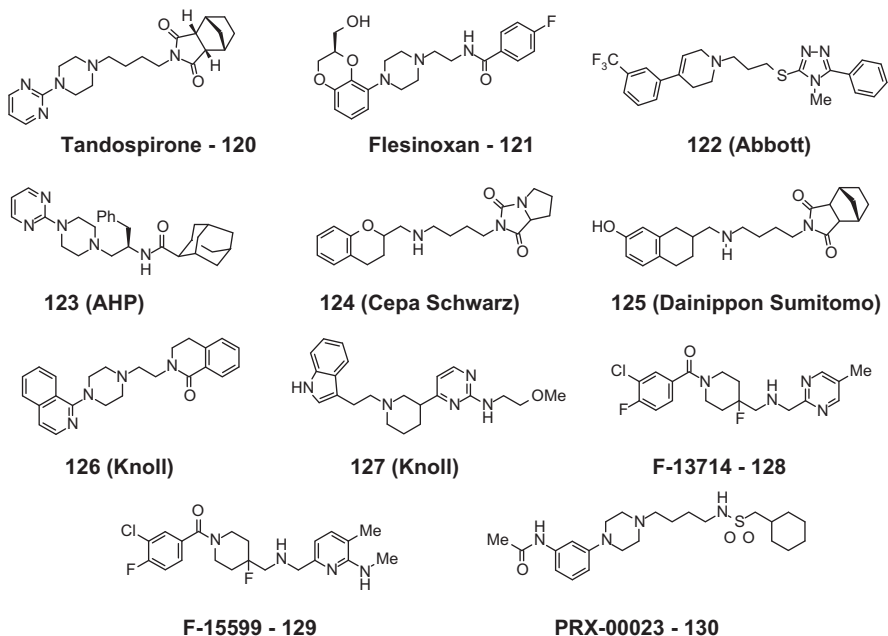
Predix pharmaceuticals have developed a series of arylpiperazino sulfonamides from which PRX-00023 (**130**) was selected. PRX-00023 (**130**) has high affinity for 5-HT<sub>1A</sub> (K<sub>i</sub>, 5–11 nM) and selectivity towards other 5-HT receptors, the next nearest affinity being for the 5-HT<sub>7</sub> receptor (K<sub>i</sub>, 700 nM). An SAR driver for this series was the attainment of longer half-life than buspirone and PRX-00023 (**130**) demonstrated a 3-fold greater half-life in human liver microsomes than buspirone.<sup>235</sup> In a placebo-controlled trial with PRX-00023 (**130**) for anxiety and depression, in a group of moderately depressed patients, no improvement in anxiety was noted; however, a highly significant improvement in the MADRS was found after 8 weeks.<sup>236</sup>

### 8.3.1.1 Additional Receptor Combinations with 5-HT<sub>1A</sub> Agonists

Quetiapine (**42**) has found utility in the treatment of bipolar disorder; however, its principal binding interactions (5-HT<sub>2A</sub> and D<sub>2</sub>) do not explain its effectiveness in bipolar disorder. Accordingly, to test the hypothesis that a metabolite of quetiapine could be responsible for the anti-bipolar effect, the principal quetiapine metabolite, norquetiapine (**131**) (Figure 8.16), has been profiled at a range of receptors and found to interact with NET (K<sub>i</sub>, 12 nM) and 5-HT<sub>1A</sub> (K<sub>i</sub>, 45 nM).<sup>237</sup>

These observations with respect to quetiapine (**42**) have stimulated the search for dual NET inhibitors/5-HT<sub>1A</sub> agonists for depression and Pfizer have reported a series of platforms with this combination. Compound **132** was demonstrated to be a partial agonist at 5-HT<sub>1A</sub> (K<sub>i</sub>, 21 nM) and a NET inhibitor (K<sub>i</sub>, 18 nM) with good selectivity over DAT (K<sub>i</sub>, 139 nM) and SERT (K<sub>i</sub>, 1,040 nM). Compound **132** was shown to occupy both 5-HT<sub>1A</sub> (77%) and NET (80%) receptors following 10 mg/kg sc dosing. Pfizer then elaborated the phenoxy-piperidine platform with the aim of reducing the logP in order to improve metabolic stability. This was accomplished through the introduction of a pyridine ring, which resulted in a reduction in logP of 1.1.<sup>238</sup> Compound **133** is a partial agonist with good affinity at 5-HT<sub>1A</sub> (K<sub>i</sub>, 11 nM) and comparable NET affinity (K<sub>i</sub>, 33 nM) to **132** and it has selectivity over DAT (K<sub>i</sub>, > 6,180 nM) and SERT (K<sub>i</sub>, 194 nM). Compound **133** occupies 5-HT<sub>1A</sub> (37%) and NET (75%) receptors following 10 mg/kg sc dosing.<sup>239</sup> Following on from the pyridylpiperidine series Pfizer investigated potential replacements for the piperidine moiety, including the azetidinyloxy and pyrrolidinyloxy fragments. The azetidinyloxy analogue (**134**) has reduced logP (2.8) and reduced 5-HT<sub>1A</sub> and NET affinity by comparison to **132** and **133** (5-HT<sub>1A</sub> K<sub>i</sub>, 64 nM; NET K<sub>i</sub>,





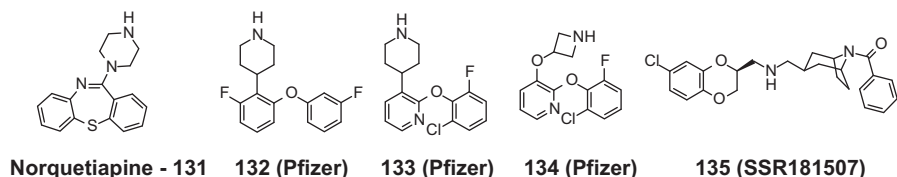
**Figure 8.16** Tandospirone, flesinoxan and 5-HT<sub>1A</sub> agonists.

73 nM) though improved selectivity over DAT ( $K_i$ , >4,260 nM) and SERT ( $K_i$ , >6,280 nM).<sup>240</sup>

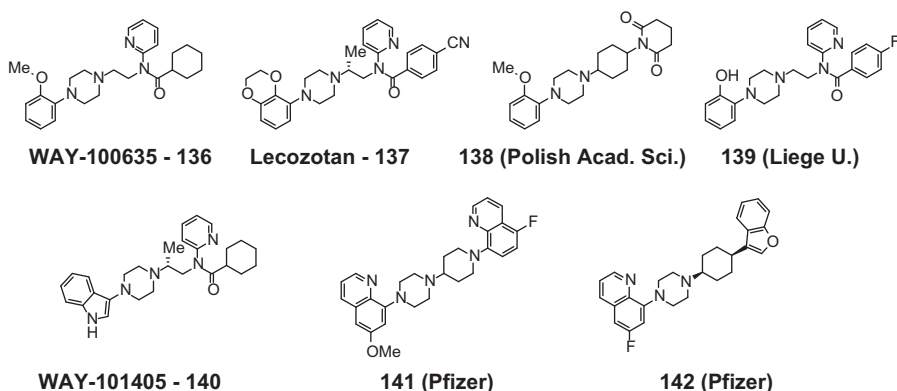
Sanofi-Aventis have identified SSR181507 (**135**), which is under development as an antipsychotic. It is a potent and selective D<sub>2</sub> antagonist and 5-HT<sub>1A</sub> agonist (D<sub>2</sub>  $K_i$ , 7.5 nM; 5-HT<sub>1A</sub>  $K_i$ , 4.5 nM). SSR181507 (**135**) has a demonstrable antidepressant signature as evidenced by activity in models of cognition and its EEG profile.<sup>241,242</sup>

### 8.3.2 5-HT<sub>1A</sub> Antagonists

The action of antagonists at the pre-synaptic 5-HT<sub>1A</sub> auto-receptor in stimulating 5-HT release was detailed in Section 8.2.1; however, 5-HT<sub>1A</sub> antagonists have additional pharmacology, which includes enhancement of the release of both glutamate and acetylcholine.<sup>243,244</sup> This additional pharmacology is significant as both glutamate and acetylcholine neurotransmission are impaired in Alzheimer's disease.<sup>245</sup> Depression in patients with Parkinson's disease has also been studied. In these patients a higher density of 5-HT<sub>1A</sub> receptors has been found *post mortem*. This has led to the suggestion of the potential utility of 5-HT<sub>1A</sub> antagonists as adjunct therapy for depression and dementia.<sup>246</sup> The potential for 5-HT<sub>1A</sub> antagonist SSRI combination therapy and the developments related to acetylcholine and glutamate pharmacologies has thus stimulated recent interest in the identification of selective 5-HT<sub>1A</sub> antagonists.<sup>247</sup>



**Figure 8.17** Norquetiapine, norepinephrine/5-HT<sub>1A</sub> agonists and dopamine D<sub>2</sub>/5-HT<sub>1A</sub> agonists.



**Figure 8.18** Lecozotan, WAY-101405 and 5-HT<sub>1A</sub> antagonists.

The development of selective 5-HT<sub>1A</sub> antagonists was advanced by the discovery of WAY-100635 (**136**) (Figure 8.17), which has proven to be a useful tool for *in vivo* experimentation. While WAY-100635 (**136**) was not developed, it is the template for a number of 5-HT<sub>1A</sub> antagonists. The first of these was lecozotan (**137**), which maintained the pyridyl and piperazine moieties of WAY-100635 (**136**) but replaced the methoxyphenyl ring with a dihydro-1,4-benzodioxine ring. Additionally the cyclohexyl amide was modified to incorporate a 4-cyanophenyl ring. Lecozotan (**137**) has high affinity for 5-HT<sub>1A</sub> ( $K_i$ , 1.6 nM), is a full antagonist and in a PET study the 5-HT<sub>1A</sub> receptor occupancy has been determined to be 50–60% following a single dose of 5 mg in elderly patients.<sup>248,249</sup>

Researchers at the Polish Academy of Science rigidified a two carbon linker to a piperidine-dione and converted a common 5-HT<sub>1A</sub> agonist motif to a high-affinity antagonist, **138**, which has 5-HT<sub>1A</sub>  $K_i$  of 3.4 nM (Figure 8.18).<sup>250</sup>

The difficulties inherent in attempting to obtain selective post-synaptic antagonists were also manifest in this SAR, as analogues from the series varied between pre-synaptic 5-HT<sub>1A</sub> agonist- and post-synaptic 5-HT<sub>1A</sub> antagonist-function.<sup>250</sup>

Following observations of an enhanced PET imaging signal with demethylated WAY-100635 (**136**), workers at the University of Liege developed the

phenol **139** as a 5-HT<sub>1A</sub> antagonist. Compound **139** potently inhibited 5-HT<sub>1A</sub> (100% at 10<sup>-6</sup> M) and has 100-fold higher affinity at 5-HT<sub>1A</sub> than the next highest affinity receptor, the alpha-1 adrenergic receptor.<sup>251</sup>

Wyeth also developed WAY-101405 (**140**), which is a high-affinity 5-HT<sub>1A</sub> antagonist ( $K_i$  versus 5-HT<sub>1A</sub>, 1.13 nM and  $K_b$ , 1.27 nM) for cognitive dysfunction. WAY-101405 (**140**) is orally bioavailable and is active in multiple rodent models of memory and learning.<sup>243</sup>

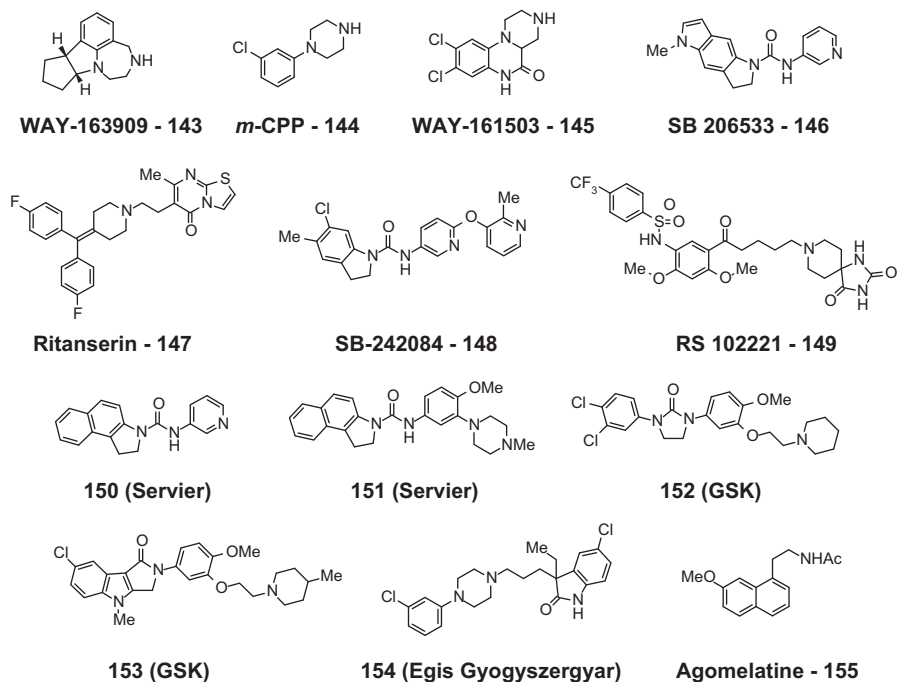
In an attempt to develop new non-amide 5-HT<sub>1A</sub> antagonists Pfizer have developed **141**, which shares a rigid structure analogous to **138**; however, **141** has a 5-fluoroquinoline ring in place of an aryl amide or cyclohexyl imide. Compound **141** has high affinity for 5-HT<sub>1A</sub> ( $K_i$ , 0.4 nM) and is a full antagonist but suffers from high *in vitro* metabolism. Despite its metabolic limitations **141** was active in an array of 5-HT<sub>1A</sub> related tests of learning and memory and also reversed fluoxetine- (**4**) induced sexual dysfunction in rats. Analogues from the quinoline series were demonstrated to have enhanced metabolic stability.<sup>252</sup> The cyclohexyl analogues of **141** have also been disclosed and exemplified as very high-affinity 5-HT<sub>1A</sub> antagonists and compound **142** has  $K_i$  of 0.11 nM. Examples from within this series also had modest SERT affinity.<sup>253</sup>

### 8.3.3 5-HT<sub>2C</sub> Agonists and Antagonists

Rationalizing the effects of agonists and antagonists at the 5-HT<sub>2C</sub> receptor is complicated by the conflicting claims of the therapeutic utility of both agonists and antagonists in the treatment of depression.<sup>254,255</sup>

The search for 5-HT<sub>2C</sub> agonists has largely been dominated by the search for anti-obesity agents with sporadic reports of the utility of agonists in the treatment of psychosis.<sup>255</sup> Thus WAY-163909 (**143**) (Figure 8.19), a selective 5-HT<sub>2C</sub> agonist ( $K_i$  versus 5-HT<sub>2C</sub>, 11 nM; EC<sub>50</sub>, 8 nM; E<sub>max</sub> 90%), was shown to be effective in both the FST and the bulbectomy models of depression. In the latter test, reduction in hyperactivity was noted at 3 mg/kg ip. Additionally, support for the utility of 5-HT<sub>2C</sub> agonists as antidepressants has been provided by the 5-HT<sub>2C</sub> agonist *meta*-chlorophenylpiperazine (*m*-CPP, **144**) in a clinical trial in elderly depressed patients.<sup>256</sup> Further evidence for the intermediacy of the 5-HT<sub>2C</sub> receptor in depression treatment is the prevention of the action of both the 5-HT<sub>2C</sub> agonist WAY-161503 (**145**) and fluoxetine in the FST by the 5-HT<sub>2C</sub> antagonist SB 206533 (**146**).<sup>257</sup>

Support for a 5-HT<sub>2C</sub> antagonist approach to the treatment of depression comes from the utility of clinically effective antidepressants that demonstrate 5-HT<sub>2C</sub> receptor affinity. For example, the high-affinity 5-HT<sub>2A/2C</sub> antagonist ritanserin (**147**) (5-HT<sub>2A</sub> p $K_i$ , 9.6; 5-HT<sub>2C</sub> p $K_i$ , 9.6) has been shown to be an effective antidepressant in a clinical trial and its 5-HT<sub>2C</sub> affinity has been invoked to rationalize its clinical effectiveness.<sup>254,258,259</sup> Furthermore the co-administration of the 5-HT<sub>2C</sub> antagonist SB-242084 (**148**) with citalopram has been shown to acutely augment the increase in synaptic 5-HT.<sup>260</sup> The same authors demonstrate that synergistic releases of 5-HT were also effected with a second 5-HT<sub>2C</sub> antagonist RS 102221 (**149**).<sup>261</sup>



**Figure 8.19** Agomelatine, 5-HT<sub>2C</sub> agonists and antagonists.

Servier was an early entrant into this field and reported a series of pyridinylcarbamoylindolines (*e.g.* **150**) as 5-HT<sub>2C</sub> antagonists for the treatment of anxiety.<sup>262</sup> The original pyridine series of Servier was subsequently elaborated to include an *ortho*-methoxypiperazinyl moiety (**151**), which was primarily a 5-HT<sub>2C</sub> antagonist ( $pK_i = 8.5$ ) but also possessed  $\alpha$ -2 adrenergic cross-reactivity.<sup>263</sup> GSK developed two series of alkoxyethyl piperidines that shared pharmacophoric elements with the Servier compounds. Of note is the imidazolone moiety, which was equivalent to the substituted urea in **151**. Developed as an anxiolytic, **152** is a potent 5-HT<sub>2C</sub> antagonist ( $pK_i$ , 9.1), which reversed an *m*-CPP- (**144**) induced hypolocomotion assay at 10 mg/kg po.<sup>264</sup> Subsequent SAR development of the **132** platform led to **153**, which, while being a weaker 5-HT<sub>2C</sub> antagonist ( $pK_i$ , 8.5), had improved druggability characteristics.<sup>265</sup> Egis Gyogyszergyar developed a structurally different class of 5-HT<sub>2C</sub> antagonists; thus, a series of piperazinopropyl indoles typified by **154** were reported to have a 5-HT<sub>2C</sub>  $K_i$  of < 50 nM. The Egis Gyogyszergyar series was not further characterized but this class of compounds has structural elements in common with 5-HT<sub>2A</sub> antagonists (*cf.* trazadone) and could be expected to show 5-HT<sub>2A</sub> cross-reactivity.<sup>266</sup>

A 5-HT<sub>2C</sub> antagonist that is also an agonist at both the melatonin-1 and melatonin-2 receptors and that has been approved for the treatment of depression in Europe is agomelatine (**155**). Agomelatine has modest affinity for

5-HT<sub>2C</sub> (pK<sub>i</sub> 6.2) and over 100-fold higher affinity at the melatonin-1 and melatonin-2 receptors.<sup>267</sup> The mechanism of action of agomelatine is unclear but it is reported to normalize circadian rhythm and this may contribute to its effectiveness.<sup>268</sup>

The need for improved therapies for neurological and psychiatric diseases is increasing and the recent recognition of the extent of the burden of disability in Europe underpins the pressure to develop new therapies.<sup>269</sup> Despite the substantial effort that has been applied to discover novel neurotherapeutics, an SRI with enhanced effectiveness or improved side-effect profile has yet to emerge. The clinical utility of the combination SRI/5-HT<sub>1A</sub> agonist vilazodone (**24**) is being established and developments with TRUIs and combinations with 5-HT<sub>2A</sub> or 5-HT<sub>3</sub> are awaited, as is the emergence of a selective post-synaptic 5-HT<sub>1A</sub> agonist.

## References

1. WHO Global Burden of Disease. [http://www.who.int/healthinfo/global\\_burden\\_disease/2004\\_report\\_update/en/index.html](http://www.who.int/healthinfo/global_burden_disease/2004_report_update/en/index.html).
2. J. Alonso, M. C. Angermeyer, S. Bernert, R. Bruffaerts, T. S. Brugha, H. Bryson, G. de Girolamo, R. Graaf, K. Demyttenaere, I. Gasquet, J. M. Haro, S. J. Katz, R. C. Kessler, V. Kovess, J. P. Lepine, J. Ormel, G. Polidori, L. J. Russo, G. Vilagut, J. Almansa, S. Arbabzadeh-Bouchez, J. Autonell, M. Bernal, M. A. Buist-Bouwman, M. Codony., A. Domingo-Salvany, M. Ferrer, S. S. Joo, M. Martinez-Alonso, H. Matschinger, F. Mazzi, Z. Morgan, P. Morosini, C. Palacin, B. Romera, N. Taub and W. A. M. Vollebergh, *Acta Psychiatr. Scand. Suppl.*, 2004, **420**, 21.
3. S. B. Guze and E. Robins, *Br. J. Psychiatry*, 1970, **117**(539), 437.
4. P. Mourilhe and P. E. Stokes, *Drug Safety*, 1998, **18**(1), 57.
5. W. K. Goodman, L. H. Price, S. A. Rasmussen, P. L. Delgado, G. R. Heninger and D. S. Charney, *Arch. Gen. Psychiatry*, 1989, **46**, 36.
6. G. D. Tollefson, A. H. Rampey, J. H. Potvin, M. A. Jenike, A. J. Rush, R. A. Kominguez, L. M. Koran, M. K. Shear, W. Goodman and L. A. Genduso, *Arch. Gen. Psychiatry*, 1994, **51**(7), 559.
7. M. A. Jenike, L. Baer, P. Summergrad, W.E. Minichiello, A. Holland and R. Seymour, *Am. J. Psychiatry*, 1990, **147**(7), 923.
8. J. Zohar and R. Judge, *Br. J. Psychiatry*, 1996, **169**(4), 468.
9. A. L. Montejo-Gonzalez, G. Llorca, J. A. Izquierdo, A. Ledesma, M. Bousono, A. Calcedo, J. L. Carrasco, J. Ciudad, E. Daniel, J. De la Gandara, J. Derecho, M. Franco, M. J. Gomez, J. A. Macias, T. Martin, V. Perez, J. M. Sanchez, S. Sanchez and E. Vicens, *J. Sex Marital Ther.*, 1997, **23**(3), 176.
10. M. J. Dresser, D. Desai, S. Gidwani, A. D. Seftel and N. B. Modi, *Int. J. Impotence Res.*, 2006, **18**(1), 104.
11. E. Hollander, C. M. DeCaria, J. N. Finkell, T. Begaz, C. M. Wong and C. Cartwright, *Biol. Psychiatry*, 2000, **47**(9), 813.

12. S. H. Sindrup, L. F. Gram, K. Brosen, O. Eshoj and E. F. Mogensen, *Pain*, 1990, **42**(2), 135.
13. O. Brawman-Mintzer, R. G. Knapp, M. Rynn, R. E. Carter and K. Rickels, *J. Clin. Psychiatry*, 2006, **67**(6), 874.
14. J. C. Ballenger, D. E. Wheadon, M. Steiner, W. Bushnell and I. P. Gergel, *Am. J. Psychiatry*, 1998, **155**(1), 36.
15. D. J. Stein, S. Seedat, G. J. van der Linden and N. Zungu-Dirwayi, *Int. Clin. Psychopharmacol.*, 2000, **15**(2), S31.
16. M. Fava and K. G. Davidson, *Psychiatr. Clin. North Am.*, 1996, **19**(2), 179.
17. M. J. Millan, *Neurotherapeutics*, 2009, **6**(1), 53.
18. R. J. Seo, H. MacPherson and A. H. Young, *Pharmaceuticals*, 2010, **3**, 3522.
19. R. W. Fuller, *Life Sci.*, 1994, **55**(3), 163.
20. P. Blier and C. De Montigny, *J. Neurosci.*, 1983, **3**(6), 1270.
21. S. A. Montgomery, S. J. Rani, R. McAuley, D. Roy and D. B. Montgomery, *Acta Psychiatr. Scand. Suppl.*, 1981, **290**, 219.
22. D. Verge, G. Daval, A. Patey, H. Gozlan, S. El Mestikawy and M. Hamon, *Eur. J. Pharmacol.*, 1985, **113**(3), 463.
23. S. Hjorth, *J. Neurochem.*, 1993, **60**(2), 776.
24. P. Blier and R. Bergeron, *J. Clin. Psychopharmacol.*, 1995, **15**, 217.
25. P. Blier and R. Bergeron, *J. Clin. Psychopharmacol.*, 1998, **59**(5), 16.
26. D. J. Koch and V. P. Rocco, 1997, *PCT Int. Appl.*, WO1997048698.
27. K. Takeuchi, T. J. Kohn, N. A. Honigschmidt, V. P. Rocco, P. G. Spinazze, D. J. Koch, D. L. Nelson, B. D. Wainscott, L. J. Ahmad, J. Shaw, P. G. Threlkeld and D. T. Wong, *Bioorg. Med. Chem. Lett.*, 2003, **13**(11), 1903.
28. A. M. Oficialdegui, J. Martinez, S. Perez, B. Heras, M. Irurzun, J. A. Palop, R. Tordera, B. Lasheras, J. Del Rio and A. Monge, *Farmaco*, 2000, **55**(5), 345.
29. E. K. Moltzen, I. Mikkelsen and C. Krog-Jensen, *PCT Int. Appl.*, WO1999067237, 1999.
30. E. K. Moltzen, J. K. Perregaard, I. Mikkelsen and P. G. Smith, *PCT Int. Appl.*, WO199905140, 1999.
31. D. E. Evrard, U. S. Shah and G. P. Stack, *PCT Int. Appl.*, WO2004024723, 2004.
32. G. P. Stack, D. E. Evrard and U. S. Shah, *PCT Int. Appl.*, WO2004024732, 2004.
33. M. Tran and G. P. Stack, *PCT Int. Appl.*, WO2002072587, 2002.
34. D. Zhou, G. P. Stack, J. Lo, A. A. Failli, D. A. Evrard, B. L. Harrison, N. T. Hatzenbuehler, M. Tran, S. Croce, S. Yi, J. Golembieski, G. A. Hornby, M. Lai, Q. Lin, L. E. Schechter, D. L. Smith, A. D. Shilling, C. Huselton, P. Mitchell, C. E. Beyer and T. H. Andree, *J. Med. Chem.*, 2009, **52**(15), 4955.
35. N. T. Hatzenbuehler, R. Baudy, D. A. Evrard, A. Failli, B. L. Harrison, S. Lenicek, R. E. Mewshaw, A. Saab, U. Shah, J. Sze, M. Zhang, D. Zhou, M. Chlenov, M. Kagan, J. Golembieski, G. Hornby, M. Lai, D. L. Smith,

- K. M. Sullivan, L. E. Schechter and T. H. Andree, *J. Med. Chem.*, 2008, **51**(21), 6980.
36. N. T. Hatzenbuehler, D. A. Evrard, B. L. Harrison, D. Huryn, J. Inghrim, C. Kraml, J. F. Mattes, R. E. Mewshaw, D. Zhou, G. Hornby, Q. Lin, D. L. Smith, K. M. Sullivan, L. E. Schechter, C. E. Beyer and T. H. Andree, *J. Med. Chem.*, 2006, **49**(15), 4785.
37. K. M. Boy, M. Dee, J. Yevich, J. Torrente, Q. Gao, L. Iben, A. Stark and R. J. Mattson, *Bioorg. Med. Chem. Lett.*, 2004, **14**(17), 4467.
38. A. Nishiyama, M. Bougauchi, M. Minoguchi, Y. Morio and T. Horikawa, *PCT Int. Appl.*, WO2002042297, 2002.
39. M. Rottlaender, E. K. Moltzen, I. Mikkelsen, T. Ruhland, K. Andersen and C. Krog-Jensen, *PCT Int. Appl.*, WO2003002556, 2003.
40. M. Rottlaender, E. K. Moltzen, I. Mikkelsen, T. Ruhland, K. Andersen and C. Krog-Jensen, *PCT Int. Appl.*, WO2003002552, 2003.
41. S. M. Bromidge, B. Bertani, B. Borriello, F. Manuela, G. Stefania, L. J. Gordon, E. Granci, M. Hill, H. R. Marshall, L. P. Stasi, V. Zucchelli, G. Merlo, A. Vesentini, J. M. Watson and L. Zonzini, *Bioorg. Med. Chem. Lett.*, 2008, **18**(20), 5653.
42. F. Herold, A. Chodkowski, L. Izbicki, J. Turlo, M. Dawidowski, J. Kleps, G. Nowak, K. Stachowicz, M. Dybala, A. Siwek, A. P. Mazurek, P. A. Mazurek and F. Plucinski, *Eur. J. Med. Chem.*, 2011, **46**(1), 142.
43. P. Blier and N. M. Ward, *Biol. Psychiatry*, 2003, **53**(3), 193.
44. P. Blier and C. De Montigny, *J. Clin. Psychopharmacol.*, 1990, **10**(3), 13S.
45. J. Dong, C. De Montigny and P. Blier, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1997, **356**(3), 303.
46. S. M. Stahl, L. Kaiser, J. Roeschen, J. M. K. Hesselink and J. Orazem, *Int. J. Neuropsychopharmacol.*, 1998, **1**(1), 11.
47. P. Blier, R. Bergeron and C. De Montigny, *Neuropsychopharmacology*, 1997, **16**(5), 333.
48. T. Heinrich, H. Boettcher, R. Gericke, G. D. Bartoszyk, S. Anzali, C. A. Seyfried, H. E. Greiner and C. van Amsterdam, *J. Med. Chem.*, 2004, **47**(19), 4684.
49. G. Bartoszyk and E. Sedman, *PCT Int. Appl.*, WO2002039989, 2002.
50. Z. A. Hughes, K. R. Starr, C. J. Langmead, G. D. Bartoszyk, J. J. Hagan, D. N. Middlemiss and L. A. Dawson, *Eur. J. Pharmacol.*, 2005, **510**, 49.
51. L. A. Dawson and J. M. Watson, *CNS Neuroscience & Therapeutics*, 2009, **15**, 107.
52. K. Rickels, M. Athanasiou, D. S. Robinson, M. Gibertini, H. Whalen and C. R. Reed, *J. Clin. Psychiatry*, 2009, **70**(3), 326.
53. M. Zhang, C. J. Stanton, N. T. Hatzenbuehler and B. L. Harrison, *PCT Int. Appl.*, WO2008080120, 2008.
54. J. M. Bartolome-Nebreda and J. I. Andres-Gil, *PCT Int. Appl.*, WO2004106346, 2004.
55. O. Barba, G. J. Dawson, T. M. Krulle, R. J. Rowley, D. Smyth and G. H. Thomas, *PCT Int. Appl.*, WO2006085118, 2006.
56. D. Hoyer and D. N. Middlemiss, *Trends Pharmacol. Sci.*, 1989, **10**(4), 130.



57. H. Rollema, T. Clarke, J. S. Sprouse and D. W. Schulz, *J. Neurochem.*, 1996, **67**(5), 2204.
58. C. D. Walker, D. T. Wong and Y. C. Xu, *PCT Int. Appl.*, WO1999020621, 1999.
59. L. Matzen, C. van Amsterdam, H. Greiner, W. Rautenberg, H. Boettcher, G. Bartoszyk and J. Harting, *Ger. Offen.*, DE199919756036, 1999.
60. L. Matzen, C. van Amsterdam, W. Rautenberg, H. E. Greiner, J. Harting, C. A. Seyfried and H. Boettcher, *J. Med. Chem.*, 2000, **43**(6), 1149.
61. I. Pullar, J. R. Boot, S. L. Carney, M. L. Cohen, E. M. Colvin, R. G. Conway, C. L. Hardy, V. L. Lucaites, D. L. Nelson, K. W. Schenck, R. Tomlinson and S. Wedley, *Eur. J. Pharmacol.*, 2001, **432**(1), 9.
62. S. N. Mitchell, R. G. Greenslade and J. Cooper, *Eur. J. Pharmacol.*, 2001, **432**(1), 19.
63. G. H. Timms, J. R. Boot, R. J. Broadmore, S. L. Carney, J. Cooper, J. D. Findlay, J. Gilmore, S. N. Mitchell, N. A. Moore, I. Pullar, G. J. Sanger, R. Tomlinson, B. B. Tree and S. Wedley, *Bioorg. Med. Chem. Lett.*, 2004, **14**(10), 2469.
64. A. Torrado, C. Lamas, J. Agejas, A. Jimenez, N. Diaz, J. Gilmore, J. Boot, J. Findlay, L. Hayhurst, L. Wallace, R. Broadmore and R. Tomlinson, *Bioorg. Med. Chem.*, 2004, **12**(20), 5277.
65. A. B. Bueno, J. Gilmore, J. Boot, R. Broadmore, J. Cooper, J. Findlay, L. Hayhurst, A. Marcos, C. Montero, S. Mitchell, G. Timms, R. Tomlinson, L. Wallace and L. Walton, *Bioorg. Med. Chem. Lett.*, 2007, **17**(12), 3344.
66. D. S. Baldwin, C. J. Hawley, R. T. Abed, B. P. Maragakis, J. Cox, S. A. Buckingham, G. H. Pover and A. Ascher, *J. Clin. Psychiatry*, 1996, **57**(2), 46.
67. A. Feiger, A. Kiev, R. K. Shrivastava, P. G. Wisselink and C. S. Wilcox, *J. Clin. Psychiatry*, 1996, **57**(2), 53.
68. R. Armitage, K. Yonkers, D. Cole and J. A. Rush, *J. Clin. Psychopharmacol.*, 1997, **17**(3), 161.
69. J. P. Feighner and W. F. Boyer, *Psychopharmacology*, 1988, **95**, S50.
70. R. Fontaine, *Clin. Neuropharmacol.*, 1993, **16**(3), S45.
71. G. I. Papakostas and M. Fava, *Eur. Psychiatry*, 2007, **22**(7), 444.
72. I. A. Pullar, S. L. Carney, E. M. Colvin, V. L. Lucaites, D. L. Nelson and S. Wedley, *Eur. J. Pharmacol.*, 2000, **407**(1/2), 39.
73. M. Giannangeli, N. Cazzolla, M. R. Luperini, M. Magnani, M. Mabilia, G. Picconi, M. Tomaselli and L. Baiocchi, *J. Med. Chem.*, 1999, **42**(3), 336.
74. J. Feighner, S. D. Targum and M. E. Bennett, *J. Clin. Psychiatry*, 1998, **59**, 246.
75. H. Mashiko, S.-I. Niwa, H. Kumashiro, Y. Kaneko, S. Suzuki, Y. Numata, R. Horikoshi and Y. Watanabe, *Psychiatry Clin. Neurosci.*, 1999, **53**(2), 193.
76. G. J. Marek, L. L. Carpenter, C. J. McDougale and L. H. Price, *Neuropsychopharmacology*, 2003, **28**, 402.

77. N. M. Richtand, J. A. Welge, A. D. Logue, P. E. Keck, S. M. Strakowski and R. K. McNamara, *Neuropsychopharmacology*, 2007, **32**(8), 1715.
78. K. Hatanaka, T. Nomura, K. Hidaka, H. Takeuchi, S. Yatsugi, M. Fujii and T. Yamaguchi, *Neuropharmacology*, 1996, **35**(11), 1621.
79. S. Mente, R. Gallaschun, A. Schmidt, L. Lebel, M. Vanase-Frawley and A. Fliri, *Bioorg. Med. Chem. Lett.*, 2008, **18**(23), 6088.
80. S. Y. Kang, E.-J. Park, W.-K. Park, H. J. Kim, G. Choi, M. E. Jung, J. H. Seo, M. J. Kim, A. N. Pae, J. Kim and J. Lee, *Bioorg. Med. Chem.*, 2010, **18**(16), 6156.
81. P. G. Mcmanis and N. J. Talley, *CNS Drugs*, 1997, **8**(5), 394.
82. J. Greist, R. K. McNamara, C. H. Mallinckrodt, J. N. Rayamajhi and J. Raskin, *Clin. Ther.*, 2004, **26**(9), 1446.
83. M. Matsumoto, M. Yoshioka, H. Togashi, M. Tochihiro, T. Ikeda and H. Saito, *J. Pharm. Exp. Ther.*, 1995, **272**(3), 1044.
84. I. Angel, H. Schoemaker, M. Prouteau, M. Garreau and S. Z. Langer, *Eur. J. Pharmacol.*, 1993, **232**, 139.
85. M. Agnel, H. Esnaud, S. Z. Langer and D. Graham, *Biochem. Pharmacol.*, 1996, **51**(9), 1145.
86. A. Lucchelli, M. G. Santagostino-Barbone, A. Barbieri, S. M. Candura and M. Tonini, *Brit. J. Pharmacol.*, 1995, **114**, 1017.
87. N. Moore, M. Dragheim and A. Batra, *PCT Int. Appl.*, WO2009062517, 2009.
88. B. Bang-Andersen, T. Ruhland, M. Jorgensen, G. Smith, K. Frederiksen, K. G. Jensen, H. Zhong, S. M. Nielsen, S. Hogg, A. Mork and T. B. Stensbol, *J. Med. Chem.*, 2011, **54**(9), 3206.
89. C. E. Beyer, Q. Lin, S. Rosenzweig-Lipson and L. E. Schechter, *Eur. J. Pharmacol.*, 2006, **539**(3), 164.
90. O. L. Pudovkina, T. I. F. H. Cremers and B. H. C. Westerink, *Synapse*, 2003, **50**(1), 77.
91. J. I. Andres, J. Alcazar, J. M. Alonso, R. M. Alvarez, M. H. Bakker, I. Biesmans, J. M. Cid, A. I. De Lucas, W. Drinkenburg, J. Fernandez, L. M. Font, L. Iturrino, X. Langlois, I. Lenaerts, S. Martinez, A. A. Megens, J. Pastor, S. Pullan and T. Steckler, *Bioorg. Med. Chem.*, 2007, **15**(11), 3649.
92. A. Gobert, M. Brocco, A. Dekeyne, B. Di Cara, G. Bouchez, F. Lejeune, R. L. Gannon and M. J. Millan, *Neuropsychopharmacology*, 2009, **34**(4), 1039.
93. P. Bernstein, C. Dantzman, R. Dedinas, L. Shen and P. Warwick, *PCT Int. Appl.*, WO2004020411, 2004.
94. G. Alvaro, F. Cardullo, R. Di Fabio, R. Giovannini, E. Piga and M. E. Tranquillini, *PCT Int. Appl.*, WO2004005256, 2004.
95. G. De Nanteuil, B. Portevin, P. Gloanec, M. J. Millan, J.-C. Ortuno, C. Mannoury la Cour and A. Gobert, *PCT Int. Appl.*, WO2006103342, 2006.
96. I. M. Anderson, *Depress. Anxiety*, 1998, **7**(1), 11.
97. I. M. Anderson and B. M. Tomenson, *J. Psychopharmacol.*, 1994, **8**(4), 238.
98. M. Briley, *Hum. Psychopharmacol.*, 1998, **13**, 99.

99. C. J. Nelson, C. M. Mazure, P. I. Jatlow, M. B. Bowers and L. H. Price, *Biol. Psychiatry*, 2004, **55**(3), 296.
100. G. E. M. Husbands, J. P. Yardley and E. A. Muth, *Eur. Pat. Appl.*, EP112669, 1984.
101. E. A. Muth, J. T. Haskins, J. A. Moyer, G. E. M. Husbands, S. T. Nielsen and E. B. Sigg, *Biochem. Pharmacol.*, 1986, **35**(24), 4493.
102. J. P. Yardley, G. E. M. Husbands, G. Stack, J. Butch, J. Bicksler, J. A. Moyer, E. A. Muth, T. Andree, H. Fletcher, M. N. G. James and A. R. Sielecki, *J. Med. Chem.*, 1990, **33**(10), 2899.
103. R. L. Rudolph, L. F. Fabre, J. P. Feighner, K. Rickels, R. Entsuah and A. T. Derivan, *J. Clin. Psychiatry*, 1998, **59**(3), 116.
104. C. Moret, M. Charveron, J. P. Finberg, J. P. Couzinier and M. Briley, *Neuropharmacology*, 1985, **24**(12), 1211.
105. B. Bonnaud, G. Mouzin, H. Cousse and J. F. Patoiseau, *Eur. Pat. Appl.*, EP200638, 1986.
106. B. Bonnaud, H. Cousse, G. Mouzin, M. Briley, A. Stenger, F. Fauran and J. P. Couzinier, *J. Med. Chem.*, 1987, **30**(2), 318.
107. M. Ansseau, P. Papart, B. Troisfontaines, F. Bartholome, M. Bataille, G. Charles, M. Schittecatte, P. Darimont and J. M. Devoitille, *Psychopharmacology*, 1994, **114**(1), 131.
108. D. W. Robertson, D. T. Wong and J. H. Krushinski, *Eur. Pat. Appl.*, EP273658, 1988.
109. D. T. Wong, F. P. Bymaster, D. A. Mayle, L. R. Reid, J. H. Krushinski and D. W. Robertson, *Neuropsychopharmacology*, 1993, **8**(1), 23.
110. F. P. Bymaster, E. E. Beedle, J. Findlay, P. T. Gallagher, J. H. Krushinski, S. Mitchell, D. W. Robertson, D. C. Thompson, L. Wallace and D. T. Wong, *Bioorg. Med. Chem. Lett.*, 2003, **13**(24), 4477.
111. M. Berk, A. D. du Plessis, M. Birkett and D. Richardt, *Int. Clin. Psychopharmacol.*, 1997, **12**(3), 137.
112. S. A. Montgomery, *Eur. Psychiatry*, 1997, **12**(4), 295s.
113. K. B. Thor, M. Kirby and L. Viktrup, *Int. J. Clin. Pract.*, 2007, **61**(8), 1349.
114. G. A. Whitlock, M. D. Andrews, A. D. Brown, P. V. Fish, A. Stobie and F. Wakenhut, *Top. Med. Chem.*, 2009, **4**, 53.
115. J. R. Boot, G. Brace, C. L. Delatour, N. Dezutter, J. Fairhurst, J. Findlay, P. T. Gallagher, I. Hoes, S. Mahadevan, S. N. Mitchell, R. E. Rathmell, S. J. Richards, R. G. Simmonds, L. Wallace and M. A. Whatton, *Bioorg. Med. Chem. Lett.*, 2004, **14**(21), 5395.
116. S. L. Boulet, S. A. Filla, P. T. Gallagher, K. J. Hudziak, A. M. Johansson, R. E. Karanjawala, J. J. Masters, V. Matassa, B. M. Mathes, R. E. Rathmell, M. A. Whatton and C. N. Wolfe, *PCT Int. Appl.*, WO2004043931, 2004.
117. S. L. Boulet, A. M. Johansson and S. M. Smith, *PCT Int. Appl.*, WO2005092835, 2005.
118. E. A. Muth., J. A. Moyer., J. T. Haskins, T. H. Andree and G. E. M. Husbands, *Drug Dev. Res.*, 1991, **23**(2), 191.

119. S. M. Shah, M. B. Fawzi, P. Raveendranath and E. C. Ehrnsperger, *US Pat. Appl.*, US20070015791, 2007.
120. S. M. Shah., E. C. Ehrnsperger, R. W. Saunders, M. B. Fawzi., R. J. Galante and G. T. Whiteside, *US Pat. Appl.*, US20070015828, 2007.
121. S. M. Shah, M. B. Fawzi, E. C. Ehrnsperger and P. Raveendranath, *US Pat. Appl.*, US20070015824, 2007.
122. P. V. Fish, M. J. Fray, D. J. Lovering, A. Stobie, F. Wakenhut and G. A. Whitlock, *PCT Int. Appl.*, WO2004110995, 2004.
123. F. Wakenhut, G. A. Allan, P. V. Fish, M. J. Fray, A. C. Harrison, R. McCoy, S. C. Phillips, A. Stobie, D. Westbrook, S. L. Westbrook and G. A. Whitlock, *Bioorg. Med. Chem. Lett.*, 2009, **19**(17), 5078.
124. N. J. F. Dreyfus, S. A. Filla, A. M. Johansson, T. J. Masquelin, J. A. Shah, E. G. Tromiczak and M. W. Walter, *PCT Int. Appl.*, WO2010117979, 2010.
125. E. L. Stangeland, P. Van Dyke, D. R. Saito, J. Schmidt, L. J. Patterson, T. J. Church and A. Hughes, *PCT Int. Appl.*, WO2010120910, 2010.
126. E. L. Stangeland, J. Schmidt, D. R. Saito, A. Hughes and L. J. Patterson, *PCT Int. Appl.*, WO2011011231, 2011.
127. M. M. Bruendl, K. L. Greene, R. A. Jennings, S. E. Lazerwith, J. Nahra, P. M. O'Brien, K. S. Para and S. M. Sheehan, *US Pat. Appl.*, US20070142389, 2007.
128. J. M. Fray, P. V. Fish, G. A. Allan, G. Bish, N. Clarke, R. Eccles, A. C. Harrison, J.-L. L. Net, S. C. Phillips, N. Regan, C. Sobry, A. Stobie, F. Wakenhut, D. Westbrook, S. L. Westbrook and G. A. Whitlock, *Bioorg. Med. Chem. Lett.*, 2010, **20**(12), 3788.
129. B. Bang-Andersen, A. Falldt, T. B. Stensboel, S. Miller and H. Lopez De Diego, *PCT Int. Appl.*, WO2007144006, 2007.
130. L. J. Patterson, R. Chao and M. Rapta, *PCT Int. Appl.*, WO2010056939, 2010.
131. G. I. Papakostas, *Eur. Neuropsychopharmacol.*, 2006, **16**, 391.
132. E. Brugue and E. Vieta, *Prog. Neuropsychopharmacol. Biol. Psych.*, 2007, **31**, 275.
133. S. H. Koslow, J. W. Maas, C. L. Bowden, J. M. Davis, I. Hanin and J. Javard, *Arch. Gen. Psychiatry*, 1983, **40**(9), 999.
134. P. L. Reddy, S. Khanna, M. N. Subhash, S. M. Channabasavanna and B. S. S. R. Rao, *Biol. Psychiatry*, 1992, **31**(2), 112.
135. P. Skolnick and A. S. Basile, *CNS Neurol. Disord.*, 2007, **6**, 141.
136. B. P. Guiard, M. El Mansari and P. Blier, *Curr. Drug Targets*, 2009, **10**(11), 1069.
137. L. L. Ioannides-Demos, J. Proietto and J. J. McNeil, *Drugs*, 2005, **65**(10), 1391.
138. W. J. Houlihan, L. Kelly, J. Pankuch, J. Koletar, L. Brand, A. Janowsky and T. A. Kopajtic, *J. Med. Chem.*, 2002, **45**(19), 4097.
139. G. A. Bray, G. L. Blackburn, J. M. Ferguson, F. L. Greenway, A. K. Jain, C. M. Mendel, J. Mendels, D. H. Ryan, S. L. Schwartz, M. L. Scheinbaum and T. B. Seaton, *Obes. Res.*, 1999, **7**(2), 189.

140. N. Finer, *Int. J. Obesity*, 2002, **26**(Suppl 4), S29.
141. U. Kiyoharu, N. Terutake, O. Tadashi and N. Hiroshi, *Eur. J. Pharmacol.*, 2009, **484**, 209.
142. A. J. Scheen, *Am. J. Cardiovascular Drugs*, 2010, **10**(5), 321.
143. A. S. Lippa and J. W. Epstein, *PCT Int. Appl.*, WO2002066427, 2002.
144. M. W. Walter, *Drug Dev. Res.*, 2005, **65**(3), 97.
145. Z. Chen, J. Yang and P. Skolnick, *Top. Med. Chem.*, 2009, **4**, 131.
146. G. Bonanomi, F. Micheli and S. Terreni, *PCT Int. Appl.*, WO2008074716, 2008.
147. R. Di Fabio, F. Micheli, G. Tedesco and S. Terreni, *PCT Int. Appl.*, WO2008031771, 2008.
148. G. Bonanomi and L. Giorgio, *PCT Int. Appl.*, WO2009141412, 2009.
149. F. Micheli, P. Cavanni, R. Arban, R. Benedetti, B. Bertani, M. Bettati, L. Bettelini, G. Bonanomi, S. Braggio, R. A. Checchia, S. Davalli, R. Di Fabio, E. Fazzolari, S. Fontana, C. Marchioro, D. Minick, M. Negri, B. Oliosi, K. D. Read, I. Sartori, G. Tedesco, L. Tarsi, S. Terreni, F. Visentini, A. Zocchi and L. Zonzini, *J. Med. Chem.*, 2010, **53**(6), 2534.
150. F. Micheli, P. Cavanni, D. Andreotti, R. Arban, R. Benedetti, B. Bertani, M. Bettati, L. Bettelini, G. Bonanomi, S. Braggio, R. Carletti, A. Checchia, M. Corsi, E. Fazzolari, S. Fontana, C. Marchioro, E. Merlo-Pich, M. Negri, B. Oliosi, E. Ratti, K. D. Read, M. Roscic, I. Sartori, S. Spada, G. Tedesco, L. Tarsi, S. Terreni, F. Visentini, A. Zocchi, L. Zonzini and R. Di Fabio, *J. Med. Chem.*, 2010, **53**(13), 4989.
151. P. Skolnick, P. Popik, A. Janowsky, B. Beer and A. S. Lippa, *Eur. J. Pharmacol.*, 2003, **461**(2–3), 99.
152. P. Skolnick, Z. Chen, A. Basile and J. W. Epstein, *PCT Int. Appl.*, WO2007016155, 2007.
153. P. Skolnick and Z. Chen, *PCT Int. Appl.*, WO2008153937, 2008.
154. P. Skolnick, Z. Chen and J. Yang, *US Pat. Appl.*, US20080293822, 2008.
155. P. Skolnick, P. Krieter, J. Tizzano, A. Basile, P. Popik, P. Czobor and A. Lippa, *CNS Drug Rev.*, 2006, **12**(2), 123.
156. B. F. Molino, B. Berkowitz and M. Cohen, *US Pat. Appl.*, US20060111385, 2006.
157. B. F. Molino, S. Liu, B. A. Berkowitz, P. R. Guzzo, J. P. Beck and M. Cohen, *PCT Int. Appl.*, WO2006020049, 2006.
158. B. F. Molino, S. Liu, A. Sambandam, P. R. Guzzo, M. Hu, C. Zha, K. Nacro, D. D. Manning, M. L. Isherwood, K. N. Fleming, W. Cui and R. E. Olson, *PCT Int. Appl.*, WO2007011820, 2007.
159. S. Liu, Y.-L. A. Yang, A. Sambandam, B. F. Molino and R. E. Olson, *PCT Int. Appl.*, WO2008141082, 2008.
160. P. R. Guzzo, B. F. Molino, W. Cui, S. Liu and R. E. Olson, *PCT Int. Appl.*, WO2008141081, 2008.
161. S. Liu, B. F. Molino and K. Nacro, *PCT Int. Appl.*, WO2010132442, 2010.

162. L. Shao, F. Wang, C. A. Malcolm, M. C. Hewitt, L. R. Bush, J. Ma, M. A. Varney, U. Campbell, S. R. Engel, L. W. Hardy, P. Koch and J. E. Campbell, *PCT Int. Appl.*, WO2007081857, 2007.
163. L. Shao, F. Wang, C. A. Malcolm, M. C. Hewitt, C. Michael, J. Ma, S. Ribe, M. A. Varney, U. Campbell, S. R. Engel, L. W. Hardy, P. Koch, R. Schreiber and K. L. Spear, *PCT Int. Appl.*, WO2008151156, 2008.
164. L. Shao and J. Ma, *PCT Int. Appl.*, WO2010091268, 2010.
165. L. Shao, F. Wang, C. A. Malcolm and M. C. Hewitt, *PCT Int. Appl.*, WO2010075064, 2010.
166. C. Sonesson, L. Swanson and F. Pettersson, *PCT Int. Appl.*, WO2010058018, 2010.
167. M. C. Lucas, R. J. Weikert, D. S. Carter, H.-Y. Cai, R. Greenhouse, P. S. Iyer, C. J. Lin, E. K. Lee, A. M. Madera, A. Moore, K. Ozboya, R. C. Schoenfeld, S. Steiner, Y. Zhai and S. M. Lynch, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5559.
168. D. S. Carter, M. C. Lucas, R. C. Schoenfeld and R. J. Weikert, *PCT Int. Appl.*, WO2009101018, 2009.
169. M. Kurimura, S. Taira, T. Tomoyasu, N. Ito, K. Tai, N. Takemura, T. Matsuzaki, Y. Menjo, S. Miyamura, Y. Sakurai, A. Watanabe, Y. Sakata, T. Masumoto, K. Akazawa, H. Sugino, N. Amada, S. Ohashi, T. Shinohara, H. Sasaki, C. Morita, J. Yamashita and S. Nakajima, *PCT Int. Appl.*, WO2006121218, 2006.
170. N. Ito, M. Kurimura, T. Yamauchi, C. Segawa, H. Sasaki, K. Tai, K. Arai and T. Shinohara, *PCT Int. Appl.*, WO2009145357, 2009.
171. J. Kehler, K. Juhl and A. Poeschl, *PCT Int. Appl.*, WO2008025361, 2008.
172. S. V. Kessar, S. K. Kulkarni, R. Jain, S. Trehan, S. K. Magadi, J. Das, G. Kaur, K. N. Singh, S. Lata and A. Dhir, *PCT Int. Appl.*, WO2009118765, 2009.
173. C.-E. Park, K.-H. Min, Y.-J. Shin, Y.-J. Shin, H.-J. Yoon, W. Kim, E.-J. Ryu, C.-M. Chung and H.-H. Kim, *PCT Int. Appl.*, WO2009148290, 2009.
174. D. Peters, J. P. Redrobe, G. M. Olsen and E. O. Nielsen, *PCT Int. Appl.*, WO2008074798, 2008.
175. D. Peters, E. O. Nielsen, K. S. Nielsen and G. Munro, *PCT Int. Appl.*, WO2010130620, 2010.
176. D. Peters, J. P. Redrobe, G. M. Olsen and E. O. Nielsen, *PCT Int. Appl.*, WO2008074797, 2008.
177. D. Peters, B. L. Eriksen, J. P. Redrobe, G. Munro and E. O. Nielsen, *PCT Int. Appl.*, WO2009016214, 2009.
178. B. L. Eriksen, D. Peters, B. H. Dahl, E. O. Nielsen and J. P. Redrobe, *PCT Int. Appl.*, WO2008025777, 2008.
179. B. Gopishetty, S. Hazeldine, S. Santra, M. Johnson, G. Modi, S. Ali, J. Zhen, M. Reith and A. Dutta, *J. Med. Chem.*, 2011, **54**(8), 2924.
180. J. L. Kinney, *Clin. Pharm.*, 1985, **4**(6), 625.
181. L. F. Fabre, H. K. Brodie, D. Garver and W. W. Zung, *J. Clin. Psychiatry*, 1983, **44**(5), 88.



182. F. P. Bymaster, J. S. Katner, D. L. Nelson, S. K. Hemrick-Luecke, P. G. Threlkeld, J. H. Heiligenstein, S. M. Morin, D. R. Gehlert and K. W. Perry, *Neuropsychopharmacology*, 2002, **27**(5), 699.
183. J. Yu, D. E. Mathisen, D. Burdette, D. G. Brown, C. Becker and D. Aharony, *Drug Metab. Dispos.*, 2010, **38**(1), 46.
184. S. M. Learned-Coughlin, M. Bergstrom, I. Savitcheva, J. Ascher, V. D. Schmith and B. Langstrom, *Biol. Psychiatry*, 2003, **54**, 800.
185. J. Dong and P. Blier, *Psychopharmacology*, 2001, **155**, 52.
186. S. E. Napier, M. J. Bingham and N. A. Dunbar, *PCT Int. Appl.*, WO2007063071, 2007.
187. D. Peters, J. P. Redrobe and E. O. Nielsen, *PCT Int. Appl.*, WO2009109517, 2009.
188. D. Peters, J. P. Redrobe, G. Munro and E. O. Nielsen, *PCT Int. Appl.*, WO2009016215, 2009.
189. D. Peters, J. P. Redrobe and E. O. Nielsen, *PCT Int. Appl.*, WO2010023196, 2010.
190. D. Peters, J. P. Redrobe and E. O. Nielsen, *PCT Int. Appl.*, WO2010023197, 2010.
191. P. Bernstein, D. Brown, B. T. Dembofsky, J. P. McCauley and R. Urbanek, *PCT Int. Appl.*, WO2010071575, 2010.
192. T. E. Wilens, *J. Clin. Psychiatry*, 2006, **67**(8), 32.
193. H. D. Nelson, K. K. Vesco, E. Haney, R. Fu, A. Nedrow, J. Miller, C. Nicolaidis, M. Walker and L. Humphrey, *J. Am. Med. Assoc.*, 2006, **295**(17), 2057.
194. M. A. Lapolla and H. Jones, *Am. J. Psychiatry*, 1970, **127**(3), 335.
195. B. Cusack, A. Nelson and E. Richelson, *Psychopharmacology*, 1994, **114**(4), 559.
196. G. D. Burrows, K. P. Maguire and T. R. Norman, *J. Clin. Psychiatry*, 1998, **59**(14), 4.
197. D. Eyding, M. Lelgemann, U. Grouven, M. Harter, M. Kromp, T. Kaiser, M. F. Kerekes, M. Gerken and B. Wieseler, *Br. Med. J.*, 2010, **341**, c4737.
198. L. A. Adler, T. Spencer, T. E. Brown, J. Holdnack, K. Saylor, K. Schuh, P. T. Trzepacz, D. W. Williams and D. Kelsey, *J. Clin. Psychopharmacol.*, 2009, **29**(1), 44.
199. S. Oberboersch, B. Sundermann, C. Sundermann, M. Haurand, H.-H. Hennies and E. Bijsterveld, *PCT Int. Appl.*, WO2005105759, 2005.
200. C. D. Beadle, J. Boot, N. P. Camp, N. Dezutter, J. Findlay, L. Hayhurst, J. J. Masters, R. Penariol and M. W. Walter, *Bioorg. Med. Chem. Lett.*, 2005, **15**(20), 4432.
201. J. Goldberg, A. Fensome, C. C. McComas and P. Zhang, *PCT Int. Appl.*, WO2008073958, 2008.
202. D. J. O'Neill, A. Adedoyin, P. D. Alfinito, J. A. Bray, S. Cosmi, D. C. Deecher, A. Fensome, J. Harrison, L. Leventhal, C. Mann, C. C. McComas, N. R. Sullivan, T. B. Spangler, A. J. Uveges, E. J. Trybulski, G. T. Whiteside and P. Zhang, *J. Med. Chem.*, 2010, **53**, 4511.



203. A. T. Vu, S. T. Cohn, E. A. Terefenko, W. J. Moore, P. Zhang, P. E. Mahaney, E. J. Trybulski, I. Goljer, R. Dooley, J. A. Bray, G. H. Johnston, J. Leiter and D. C. Deecher, *Bioorg. Med. Chem. Lett.*, 2009, **19**(9), 2464.
204. A. T. Vu, S. T. Cohn, P. Zhang, C. Y. Kim, P. E. Mahaney, J. A. Bray, G. H. Johnston, E. J. Koury, S. A. Cosmi, D. C. Deecher, V. A. Smith, J. E. Harrison, L. Leventhal, G. T. Whiteside, J. D. Kennedy and E. J. Trybulski, *J. Med. Chem.*, 2010, **53**(5), 2051.
205. B. P. Clark, P. T. Gallagher and H. L. Haughton, *PCT Int. Appl.*, WO2004018440, 2004.
206. P. T. Gallagher, C. Lamas-Peteira and F. J. Agejas-Chicharro, *PCT Int. Appl.*, WO2005066144, 2005.
207. M. J. Cases-Thomas, J. J. Masters, M. W. Walter, G. Campbell, L. Haughton, P. T. Gallagher, D. R. Dobson, V. Mancuso, B. Bonnier, T. Giard, T. Defrance, M. Vanmarsenille, A. Ledgard, C. White, S. Ouwerkerk-Mahadevan, F. J. Brunelle, N. A. Dezutter, C. A. Herbots, J. Y. Lienard, J. Findlay, L. Hayhurst, J. Boot, L. K. Thompson and S. Hemrick-Luecke, *Bioorg. Med. Chem. Lett.*, 2006, **16**(7), 2022.
208. G. I. Campbell, M. J. Cases-Thomas, T. Man, J. J. Masters, H. C. E. Rudyk and M. W. Walter, 2005, *PCT Int. Appl.*, WO2005047272.
209. P. V. Fish, M. C. Mackenny, A. Stobie, F. Wakenhut and G. A. Whitlock, 2005, *PCT Int. Appl.*, WO2005105100.
210. B. W. Caprathe, S. A. Glase, Z. Konstantinou, R. M. Schelkun, S. M. Sheehan, A. J. Thomas and P. Yuen, *US Pat. Appl.*, US20050096327, 2005.
211. R. M. Schelkun and P. Yuen, *US Pat. Appl.*, US20060030566, 2006.
212. J. P. Feighner and J. B. Cohn, *Neuropsychobiology*, 1989, **21**(3), 124.
213. E. C. Dimitriou and C. E. Dimitriou, *J. Clin. Psychopharmacol.*, 1998, **18**(6), 465.
214. P. J. McGrath, J. W. Stewart, F. M. Quitkin, S. Wager, S. W. Jenkins, D. G. Archibald, J. C. Stringfellow and D. S. Robinson, *J. Clin. Psychopharmacol.*, 1994, **14**(5), 347.
215. R. E. Gammans, R. F. Mayol and J. A. LaBudde, *Am. J. Med.*, 1986, **80**, 41.
216. J. L. Maurel, J.-M. Autin, P. Funes, A. Newman-Tancredi, F. C. Colpaert and B. Vacher, *J. Med. Chem.*, 2007, **50**(20), 5024.
217. A. Hamik, D. Oksenberg, C. Fischette and S. J. Peroutka, *Biol. Psychiatry*, 1990, **28**(2), 99.
218. K. Nishitsuji, H. To, Y. Murakami, K. Kodama, D. Kobayashi, T. Yamada, C. Kubo and K. Mine, *Clin. Drug Invest.*, 2004, **24**(2), 121.
219. S. Sato, K. Mizukami and T. Asada, *Int. J. Neuropsychopharmacol.*, 2007, **10**(2), 281.
220. W. Kuipers, C. G. Kruse, I. van Wijngaarden, P. J. Standaar, M. T. M. Tulp, N. Veldman, A. L. Spek and A. P. Ijzerman, *J. Med. Chem.*, 1997, **40**, 300.
221. P. Grof, R. Joffe, S. Kennedy, E. Persad, J. Syrotiuk and D. Bradford, *Int. Clin. Psychopharmacol.*, 1993, **8**(3), 167.

222. J. Van Harten, D. L. Bradford and P. De Koning, *Eur. Pat. Appl.*, EP710481, 1996.
223. D. Starck, G. Steiner, L. Szabo, B. Behl, F. J. Garcia-Ladona and L. Unger, *Ger. Offen.*, DE10109867, 2002.
224. G. Steiner, D. Starck, K. Schellhaas, L. Szabo, B. Behl, F. J. Garcia-Ladona and L. Unger, *Ger. Offen.*, DE10109866, 2002.
225. W. E. Childers, H. Fletcher, M. A.-M. Abou-Gharbia and J. P. Yardley, *PCT Int. Appl.*, WO2001034586, 2001.
226. J. Z. Del Rio, D. Frechilla-Manso, L. M. Lopez-Rodriguez, B. Benhamu-Salama, J. A. Fuentes-Cubero and M. Delgado-Wallace, *PCT Int. Appl.*, WO2004014915, 2004.
227. T. Toyoda, M. Tanaka, N. Fujibayashi, M. Maruyama, I. Saji, F. Antoku, M. Muto and M. Oda, *PCT Int. Appl.*, WO2008047839, 2008.
228. G. Steiner, K. Schellhaas, L. Szabo, B. Behl, F. J. Garcia-Ladona and L. Unger, *PCT Int. Appl.*, WO2002002529, 2002.
229. G. Steiner, K. Schellhaas, L. Szabo, B. Behl, F. J. Garcia-Ladona and L. Unger, *PCT Int. Appl.*, WO2002002568, 2002.
230. A. B. Dounay, N. S. Barta, J. A. Bikker, S. A. Borosky, B. M. Campbell, T. Crawford, L. Denny, L. M. Evans, D. L. Gray, P. Lee, E. A. Lenoir and W. Xu, *Bioorg. Med. Chem. Lett.*, 2009, **19**(4), 1159.
231. W. Koek, B. Vacher, C. Cosi, M.-B. Assie, J.-F. Patoiseau, P. J. Pauwels and F. C. Colpaert, *Eur. J. Pharmacol.*, 2001, **420**(2/3), 103.
232. L. Llado-Pelfort, M.-B. Assie, A. Newman-Tancredi, F. Artigas and P. Celada, *Brit. J. Pharmacol.*, 2010, **160**(8), 1929.
233. M.-B. Assie, L. Bardin, A. L. Auclair, E. Carilla-Durand, R. Depoortere, W. Koek, M. S. Wouter, F. C. Kleven, B. Colpaert, Vacher and A. Newman-Tancredi, *Int. J. Neuropsychopharmacol.*, 2010, **13**(10), 1285.
234. R. Depoortere, A. L. Auclair, L. Bardin, F. C. Colpaert, B. Vacher and A. Newman-Tancredi, *Eur. Neuropsychopharmacol.*, 2010, **20**(9), 641.
235. O. M. Becker, D. S. Dhanoa, Y. Marantz, D. Chen, S. Shacham, S. Cheruku, A. Heifetz, P. Mohanty, M. Fichman, A. Sharadendu, R. Nudelman, M. Kauffman and S. Noiman, *J. Med. Chem.*, 2006, **49**(11), 3116.
236. K. Rickels, S. Mathew, M. D. Banov, D. L. Zimbroff, S. Oshana, E. C. Parsons, S. R. Donahue, M. Kauffman, G. R. Iyer and J. F. Reinhard, *J. lin. Psychopharmacol.*, 2008, **28**(2), 235.
237. N. H. Jensen, R. M. Rodriguiz, M. G. Caron, W. C. Wetsel, R. B. Rothman and B. L. Roth, *Neuropsychopharmacology*, 2008, **33**, 2303.
238. D. L. Gray, W. Xu, B. M. Campbell, A. B. Dounay, N. C. Barta, S. Boroski, L. Denny, L. Evans, N. Stratman and A. Probert, *Bioorg. Med. Chem. Lett.*, 2009, **19**(23), 6604.
239. A. B. Dounay, N. S. Barta, B. M. Campbell, C. Coleman, E. M. Collantes, L. Denny, S. Dutta, D. L. Gray, D. Hou, R. Iyer, S. N. Maiti, D. F. Ortwine, A. Probert, N. C. Stratman, R. Subedi, T. Whisman, W. Xu and K. Zoski, *Bioorg. Med. Chem. Lett.*, 2010, **20**(3), 1114.

240. M. Pettersson, B. M. Campbell, A. B. Dounay, D. L. Gray, L. Xie, C. J. O'Donnell, N. C. Stratman, K. Zoski, E. Drummond, G. Bora, A. Probert and T. Whisman, *Bioorg. Med. Chem. Lett.*, 2011, **21**(2), 865.
241. D. Boulay, R. Depoortere, C. Louis, M. Lacave, M. T. Lucas and G. Griebel, *Pharmacol. Biochem. Behav.*, 2011, **97**(3), 428.
242. R. Depoortere, D. Boulay, G. Perrault, O. Bergis, M. Decobert, D. Francon, M. Jung, J. Simiand, P. Soubrie and B. Scatton, *Neuropsychopharmacology*, 2003, **28**(11), 1889.
243. W. D. Hirst, T. H. Andree, S. Aschmies, W. E. Childers, T. A. Comery, L. A. Dawson, M. Day, I. B. Feingold, S. M. Grauer, B. L. Harrison, Z. A. Hughes, J. Kao, M. G. Kelly, H. van der Lee, S. Rosenzweig-Lipson, A. L. Saab, D. L. Smith, K. Sullivan, S. J. Sukoff-Rizzo, C. Tio, M.-Y. Zhang and L. E. Schechter, *J. Pharmacol. Exp. Ther.*, 2008, **325**, 134.
244. L. E. Schechter, D. L. Smith, S. Rosenzweig-Lipson, S. J. Sukoff, L. A. Dawson, K. Marquis, D. Jones, M. Piesla, T. Andree, S. Nawoschik, J. A. Harder, M. D. Womack, J. Buccafusco, A. V. Terry, B. Hoebel, P. Rada, M. Kelly, M. Abou-Gharbia, J. E. Barrett and W. E. Childers, *J. Pharmacol. Exp. Ther.*, 2005, **314**, 1274.
245. J. H. Chin, L. Ma, D. MacTavish and J. H. Jhamandas, *J. Neurosci.*, 2007, **27**(35), 9262.
246. S. I. Sharp, C. G. Ballard, I. Ziabreva, M. A. Piggott, R. H. Perry, E. K. Perry, D. Aarsland, U. Ehrt, J. P. Larsen and P. T. Francis, *Dementia Geriatr. Cognit. Disord.*, 2008, **26**(4), 330.
247. E. Lacivita, M. Leopoldo, F. Berardi and R. Perrone, *Curr. Top. Med. Chem.*, 2008, **8**(12), 1024.
248. W. E. Childers, M. A. Abou-Gharbia, M. G. Kelly, T. H. Andree, B. L. Harrison, D. M. Ho, G. Hornby, D. M. Huryn, L. Potestio, S. J. Rosenzweig-Lipson, J. Schmid, D. L. Smith, S. J. Sukoff, G. Zhang and L. E. Schechter, *J. Med. Chem.*, 2005, **48**(10), 3467.
249. S. Raje, A. A. Patat, V. Parks, L. Schechter, A. Plotka, J. Paul and B. A. Langstrom, *Clin. Pharmacol. Ther.*, 2008, **83**(1), 86.
250. A. J. Bojarski, M. H. Paluchowska, B. Duszynska, R. Bugno, A. Klodzinska, E. Tatarczynska and E. Chojnacka-Wojcik, *Bioorg. Med. Chem.*, 2006, **14**(5), 1391.
251. C. Defraiteur, A. Plenevaux, J. Scuvee-Moreau, N. Rouchet, D. Goblet, A. Luxen and V. Seutin, *Br. J. Pharmacol.*, 2007, **152**(6), 952.
252. W. E. Childers, L. M. Havran, M. Asselin, J. J. Bicksler, D. C. Chong, G. T. Grosu, Z. Shen, M. A. Abou-Gharbia, A. C. Bach, B. L. Harrison, N. Kagan, T. Kleintop, R. Magolda, V. Marathias, A. J. Robichaud, A. L. Sabb, M.-Y. Zhang, T. H. Andree, S. H. Aschmies, C. Beyer, T. A. Comery, M. Day, S. M. Grauer, Z. A. Hughes, S. Rosenzweig-Lipson, B. Platt, C. Pulicicchio, D. E. Smith, S. J. Sukoff-Rizzo, K. M. Sullivan, A. Adedoyin, C. Huselton and W. D. Hirst, *J. Med. Chem.*, 2010, **53**(10), 4066.
253. A. M. Venkatesan, O. D. Santos, M. Asselin, G. T. Grosu, D. A. Evrard, R. E. Mewshaw, K. Meagher, *US Pat. Appl.*, US 20090054454, 2009.

254. C. B. P. Martin, M. Hamon, L. Lanfumey and R. Mongeau, *Mod. Trends Pharmacopsychiatry*, 2010, **27**, 155.
255. N. J. T. Monck and G. A. Kennett, *Progress in Medicinal Chemistry*, ed. G. Lawton and D. R. Witty, Elsevier, Amsterdam, 2008, p. 281.
256. J. A. M. Mellow, B. A. Lawlor, T. Sunderland, E. A. Mueller, S. E. Molchan and D. L. Murphy, *Biol. Psychiatry*, 1990, **28**(7), 588.
257. J. F. Cryan and I. Lucki, *J. Pharmacol. Exp. Ther.*, 2000, **295**(3), 1120.
258. D. W. Bonhaus, K. K. Weinhardt, M. Taylor, A. Desouza, P. M. McNeeley, K. Szczepanski, D. J. Fontana, J. Trinh, C. L. Rocha, M. W. Dawson, L. A. Flippin and R. M. Eglen, *Neuropharmacology*, 1997, **36**(4/5), 621.
259. D. Bakish, Y. D. Lapierre, R. Weinstein, J. Klein, A. Wiens, B. Jones, E. Horn, M. Browne, D. Bourget, A. Blanchard, C. Thibaudeau, C. Waddell and D. Raine, *J. Clin. Psychopharmacol.*, 1993, **13**(6), 409.
260. F. Sotty, J. H. A. Folgering, L. T. Brennum, S. Hogg, A. Mork, P. Hertel and T. I. F. H. Cremers, *Neuropharmacology*, 2009, **57**(1), 18.
261. T. I. H. Cremers, M. Giorgetti, F. J. Bosker, S. Hogg, J. Arnt, A. Mork, G. Honig, K.-P. Bogeso, B. H. C. Westerink, H. den Boer, H. V. Wikstrom and L. H. Tecott, *Neuropsychopharmacology*, 2004, **29**(10), 1782.
262. G. Lavielle, O. Muller, M. Millan and A. Gobert, *Eur. Pat. Appl.*, EP1348704, 2003.
263. G. Lavielle, O. Muller, M. Millan, A. Gobert and B. Di Cara, *US Pat. Appl.*, US20050059675, 2005.
264. C. J. Goodacre, S. M. Bromidge, D. Clapham, F. D. King, P. J. Lovell, M. Allen, L. P. Campbell, V. Holland, G. J. Riley, K. R. Starr, B. K. Trail and M. D. Wood, *Bioorg. Med. Chem. Lett.*, 2005, **15**(22), 4989.
265. D. Hamprecht, F. Micheli, G. Tedesco, A. Checchia, D. Donati, M. Petrone, S. Terreni and M. Wood, *Bioorg. Med. Chem. Lett.*, 2007, **17**(2), 428.
266. B. Volk, J. Barkoczy, G. Simig, T. Mezei, D. R. Kapillerne, E. Florian, I. Gacsalyi, K. Pallagi, G. Gigler, G. Levay, K. Moricz, C. Leveleki, N. Sziray, G. Szenasi, A. Egyed and G. L. Harsing, *PCT Int. Appl.*, WO2005109987, 2005.
267. M. J. Millan, A. Gobert, F. Lejeune, A. Dekeyne, A. Newman-Tancredi, V. Pasteau, J.-M. Rivet and D. Cussac, *J. Pharmacol. Exp. Ther.*, 2003, **306**(3), 954.
268. M. B. Girish, K. Bhuvana, G. Nagesh-Raju and N. Sarala, *Int. J. Pharm. Biomed. Res.*, 2010, **1**(3), 113.
269. H. U. Wittchen, F. Jacobi, J. Rehm, A. Gustavsson, M. Svensson, B. Joensson, J. Olesen, C. Allgulander, J. Alonso, C. Faravelli, L. Fratiglioni, P. Jennum, R. Lieb, A. Maercker, J. van Os, M. Preisig, L. Salvador-Carulla, R. Simon and H.-C. Steinhausen, *Eur. Neuropsychopharmacol.*, 2011, **21**(9), 655.

## CHAPTER 9

# *Neurocircuitry of Anxiety Disorders: Focus on Panic Disorder and Post-traumatic Stress Disorder*

JAMES W. MURROUGH,<sup>\*a,c,d,g</sup> DANIELA SCHILLER<sup>b,c,d,g</sup>  
AND DENNIS S. CHARNEY, MD<sup>c,d,e,f,g</sup>

<sup>a</sup> Mood and Anxiety Disorders Program, Mount Sinai School of Medicine, 1 Gustave L. Levy Place, Box 1230, New York, NY 10029, USA;

<sup>b</sup> Laboratory of Affective Neuroscience, Mount Sinai School of Medicine;

<sup>c</sup> Department of Psychiatry, Mount Sinai School of Medicine; <sup>d</sup> Department of Neuroscience, Mount Sinai School of Medicine; <sup>e</sup> Department of Pharmacology, Mount Sinai School of Medicine; <sup>f</sup> Office of the Dean, Mount Sinai School of Medicine; <sup>g</sup> Friedman Brain Institute, Mount Sinai School of Medicine.

\*Email: james.murrough@mssm.edu

## 9.1 Introduction

Anxiety disorders are exceedingly common and account for a considerable proportion of the public health burden of mental illnesses.<sup>1</sup> Anxiety and fear are universal human emotions that serve an essential role in shaping adaptive behaviour. Excessive and sustained levels of anxiety, in contrast, may lead to marked suffering and disability. This chapter will begin with an overview of the epidemiology and clinical features of anxiety disorders with a focus on two

common anxiety disorders: panic disorder and post-traumatic stress disorder (PTSD). The chapter will then review recent advances in neurocircuitry models of fear based on behavioural and molecular studies in animals and human neuroimaging studies. Neurocircuitry models of fear learning provide a platform on which to base translational and therapeutic studies in human anxiety disorders. Findings from human pharmacological challenge studies and other clinical studies in panic disorder and PTSD will then be reviewed. Finally, the chapter concludes with a brief discussion of potential novel therapeutic strategies for anxiety disorders based on the findings from preclinical and clinical studies presented.

## 9.2 Epidemiology and Clinical Features of Anxiety Disorders

### 9.2.1 Epidemiology of Anxiety Disorders

It is estimated that approximately 1 in 4 individuals in the United States with suffer from an anxiety disorder at some point in their lifetime.<sup>1</sup> Anxiety disorders, as a class, are characterized by distressing and functionally impairing anxiety, fear and related psychic and somatic symptoms that are out of proportion to any potential or perceived threat.<sup>2</sup> *The Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition – Text Revision (DSM-IV-TR) recognizes seven primary anxiety disorders: panic disorder and agoraphobia, social phobia, specific phobia, obsessive–compulsive disorder (OCD), PTSD, generalized anxiety disorder (GAD) and acute stress disorder. The lifetime prevalence of panic disorder is estimated to be 1.9% in men and 5.1% in women, while that of PTSD is 5% to 6% in men and 10% to 14% in women.<sup>1</sup> Risk factors for the development of anxiety disorders in general include female gender, lower socioeconomic status and lower years of education, a family history of anxiety disorders and certain early patterns of temperament and personality. Although the reason for this gender difference is not known, relevant factors likely include differential reporting of symptoms (under-reporting in men), as well as gender specific social, psychological and biological factors.

All of the major anxiety disorders have been shown to aggregate in families, and numerous twin studies confirm the presence of genetic, in addition to non-genetic, modes of familial transmission. Among the anxiety disorders, panic disorder has demonstrated the highest rates of familial aggregation and genetic heritability. Individuals who have a first-degree biological relative with panic disorder are eight times more likely to develop the disorder compared to the general population.<sup>3</sup> If the onset of panic disorder occurs before the age of 20, the risk in first-degree relatives is increased up to 20 times.

Events that lead to PTSD usually involve interpersonal violence such as rape or assault, exposure to life-threatening situations such as combat or accidents, or natural disasters. Victims of sexual assault are at especially high risk for

subsequent mental health problems and suicide. In a survey of 20- to 30-year-olds in a large health management organization (HMO), 39% experienced a traumatic event, and the lifetime rate of PTSD in those exposed was 24% (*i.e.* lifetime population prevalence 9%). Risk factors for exposure to traumatic events included family history of any psychiatric disorder, history of conduct disorder symptoms, male sex, extroversion and neuroticism. Risk factors for PTSD following exposure to trauma included separation from parents during childhood, family history of anxiety, pre-existing anxiety or depression, family history of antisocial behaviour, female sex and neuroticism.<sup>4</sup>

### 9.2.2 Clinical Features of Panic Disorder

Panic disorder is characterized by recurrent discrete attacks of anxiety accompanied by several somatic symptoms, such as palpitations, paresthesias, hyperventilation, diaphoresis, chest pain, dizziness, trembling and dyspnea.<sup>2</sup> Panic disorder may be accompanied by agoraphobia, which consists of excessive fear (and often avoidance) of situations, such as driving, crowded places, stores or being alone, in which escape or obtaining help would be difficult. Panic disorder usually begins with a spontaneous panic attack that often leads the individual to seek medical treatment, such as presenting to an emergency room believing that he or she is having a heart attack, stroke, “going crazy” or experiencing some other serious medical event.<sup>4</sup> Although the panic attacks themselves can be extremely distressing to patients, it is the anticipatory anxiety, avoidance and social withdrawal associated with the agoraphobia that can complicate panic disorder that is often the greatest source of functional disability.

### 9.2.3 Clinical Features of PTSD

PTSD is a syndrome that develops in a subgroup of individuals who are exposed to an extremely stressful event that, by definition, provokes fear, horror or helplessness.<sup>2</sup> PTSD is characterized by three clusters of symptoms: (1) re-experiencing of the trauma, (2) avoidance of reminders of the trauma and (3) increased physiologic arousal and startle, although individual presentation of PTSD can vary significantly in presentation and the degree of symptomatology corresponding to the individual clusters. Re-experiencing phenomena include intrusive memories, flashbacks, nightmares and psychological or physiological distress in response to trauma reminders. Intrusive memories are spontaneous, unwanted, distressing recollections of the traumatic event. Repeated nightmares contain themes of the trauma or a highly accurate and detailed recreation of the actual event(s). Flashbacks are dissociative states in which the person feels as if he or she is actually reliving the event and loses contact with their current environment, usually for a few seconds or minutes. Reactivity to trauma-related stimuli can involve intense emotional distress or physical symptoms similar to those of a panic attack when exposed to sights,



sounds, smells or events that were present during the traumatic event. Avoidance can include thoughts, feelings, situations or activities that are reminders of the trauma. Numbing might occur through amnesia for parts of the event, emotional detachment, restricted affect or loss of interest in activities. Increased arousal can include insomnia, irritability, hypervigilance (exaggerated watchfulness, feeling on guard, checking for danger), increased startle response (“jumpiness”) or impaired concentration.<sup>4</sup>

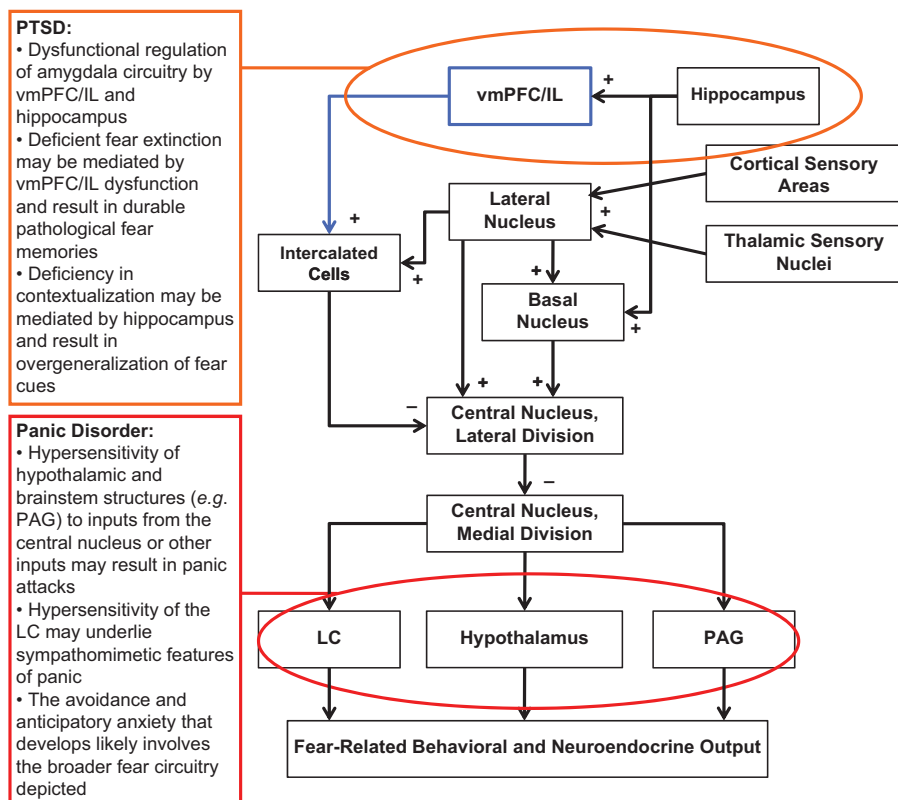
## 9.3 Advances in Neurocircuitry Models of Fear

### 9.3.1 Overview of Fear Conditioning

A wealth of preclinical research is increasing our understanding of the neural and molecular mechanisms of fear. These emerging insights are beginning to be integrated into a neurocircuitry model of normal and pathological fear that can serve as a platform for translational research aimed at discovering mechanisms and new treatments for human anxiety disorders (Figure 9.1). A classic protocol to investigate acquisition and extinction of fear in the laboratory is Pavlovian conditioning. In this protocol, a harmful event (*e.g.* a painful pinch) occurs in a reliable manner after a harmless cue (*e.g.* the sound of a bell). In this way, the innocuous cue becomes predictive of the harmful event. As a consequence there is a shift of the emotional response to the innocuous cue. Once conditioned, it is capable of triggering physiological and behavioural reactions of fear. In order to extinguish this association, the conditioned cue ought to be presented repeatedly without the harmful outcome, until eventually fear responses to the conditioned cue disappear. The harmful event is termed the Unconditioned Stimulus, or US; the harmless cue is termed the Conditioned Stimulus, or CS. Some protocols use two neutral cues, one that is paired with the US (CS+) and one that is not (CS−). The task, in this case, is learning to discriminate between the two.

### 9.3.2 The Essential Role of the Amygdala in Fear Conditioning

Pavlovian conditioning has proven to be a useful tool for investigating the underlying neural mechanisms of fear conditioning in the animal and human brain.<sup>5</sup> The amygdala is a key component of the fear system. It is built of multiple sub-structures, of which sum of computations may or may not lead to a fear response (Figure 9.1). Cortical and thalamic inputs carrying information about the US and the CS use the excitatory neurotransmitter glutamate and synapse in the lateral nucleus of the amygdala. Before the association is formed, the strong US input is necessary to activate the post-synaptic amygdala neuron. The convergence, however, of a strong US input and a relatively weak CS input leads to the enhancement of the CS input. This strengthening then allows the CS input to independently activate the post-synaptic amygdala neuron, without the co-activation of the US.<sup>6</sup>



**Figure 9.1** Schematic diagram depicting amygdala nuclei and related structures critical for fear learning and coordination of the fear response. Specific aspects of this circuitry hypothesized to be involved in PTSD or panic disorder are highlighted. In PTSD (highlighted in orange), animal and human research suggests a core deficit in the functioning of the vmPFC (the human homologue of the infralimbic cortex in rodents) leading to dysregulation of amygdala-based fear learning, in particular deficient fear extinction. The circuitry critical for fear extinction is coloured in blue. Research also implicates deficient functioning of the hippocampus in PTSD, potentially resulting in a failure to contextualize fear cues and overgeneralization of fear memories. In contrast, research implicates hypothalamic and brainstem structures (including the PAG and LC) as critical in the pathophysiology of panic disorder (highlighted in red). Overstimulation of these regions may precipitate a spontaneous panic attack while the avoidance and anticipatory anxiety that can develop as a consequence of panic attacks likely depends on the broader amygdala-based fear circuitry. See text for details. The structures in the schematic labelled intercalated cells, lateral nucleus, basal nucleus and lateral and medial divisions of central nucleus are all components of the amygdala. “+” indicates an excitatory synapse; “-” indicates an inhibitory synapse; connections that do not include a sign are modulatory in nature or the nature of the connection is not well understood. Abbreviations: IL, infralimbic cortex; LC, locus coeruleus; PAG, periaqueductal gray; vmPFC, ventromedial prefrontal cortex

The lateral nucleus can be partitioned into dorsal and ventral parts, each with a different population of neurons. Within the dorsal part there are two important populations of neurons. One population, residing at the tip of the dorsal part, is characterized by short-latency and transient responses to incoming stimuli. The other population, residing more ventrally, maintains long-latency responses throughout learning and beyond. One class of neurons, therefore, engages in learning and the other in long-term storage.<sup>7</sup> The lateral nucleus transfers information to the central nucleus, either directly or through the basal nucleus (Figure 9.1). The central nucleus is the final port through which information departs from the amygdala to the hypothalamus and the brainstem. These output projections control the expression of innate fear responses, such as freezing, and the physiological responses associated with arousal, *via* hormonal and neuromodulatory changes.<sup>8,9</sup> The central nucleus can be further partitioned into the lateral and medial subnuclei. The medial subnucleus is the very final exit way of the projections to the hypothalamus and brainstem. The lateral subnucleus keeps the medial part under continuous inhibition *via* gamma-aminobutyric acid GABAergic signalling. Only inhibition of lateral neurons allows medial neurons to promote the expression of fear. The lateral subnucleus in itself is required for fear conditioning,<sup>10</sup> suggesting that not only the lateral nucleus but also the central nucleus is participating in the acquisition of conditioned fear.

Neurons in the central nucleus are predominantly GABAergic. Within the lateral subnucleus there are two distinct populations – those that show an increase (“on” neurons) and those that show a decrease (“off” neurons) during the presentation of the CS. Both populations send inhibitory projections to the medial subnucleus. Fear response to the CS, therefore, depends on the balance between the two populations.<sup>10</sup> Other patches of GABAergic neurons lay on the border between the central, lateral and basal nuclei. These islands of inhibitory neuron are called the intercalated (ITC) cell masses, and they also exert inhibitory control over the central nucleus.<sup>11</sup> The lateral and basal amygdala nuclei send excitatory glutamatergic projections onto the ITC cells as well as directly to the central nucleus. This excitation of the ITC cells results in inhibition of the lateral division of the central nucleus, which releases the medial division from tonic inhibition, thus giving rise to the fear response.<sup>11</sup> Overall, the balance between excitatory glutamatergic and inhibitory GABAergic signalling with amygdala microcircuitry determines whether or not a fear response is elicited.

### 9.3.3 The Essential Role of the Prefrontal Cortex in Fear Extinction

What distinguishes individuals who experience a time-limited stress reaction and recover from those who manifest a potentially chronic and disabling course of PTSD or other anxiety disorder? One potentially illuminating model focuses on the process of fear extinction and posits that PTSD in particular may be

characterized by a deficit in fear extinction and/or extinction recall<sup>12–14</sup> (Figure 9.1). Fear extinction refers to the process by which a CS that previously elicited a fear reaction subsequently loses the capacity to do so. In a fear extinction paradigm, an animal first undergoes fear conditioning in which a CS-UC association is formed; this is then followed (usually the next day) by multiple presentations of the CS without the US, which leads to an eventual loss of fear responding to the CS.

An important external source of glutamatergic projections onto ITC cells within the amygdala originates from the infralimbic cortex (IL) in rodents, which is the homologue of the human ventromedial prefrontal cortex (vmPFC) (Figure 9.1).<sup>15</sup> Neurons in IL are critical to signal memory for fear extinction in the rat brain. Milad and Quirk (2002) showed that IL neurons fire to a CS during acquisition and extinction of fear, only when the rats successfully retrieved the memory on the following day.<sup>16</sup> They were able to simulate extinction memory by stimulating IL neurons together with CS presentations. In this case, rats froze to the CS, as if they underwent extinction. If the IL cortex is damaged, rats are unable to retrieve extinction memory. These data converge to suggest that IL is essential for the storage of long-term extinction memory.<sup>17,18</sup>

Extinction training potentiates IL-ITC connections, allowing the IL cortex to reduce the expression of fear.<sup>15,19</sup> The basal nucleus of the amygdala also participates in fear acquisition and extinction and contributes to the regulation of central nucleus output. Projections from the hippocampus to the basal nucleus and the IL cortex provide the contextual modulation of the expression of fear.<sup>20,21</sup> This modulation limits the expression of fear conditioning or extinction to the context in which each type of training took place.<sup>22</sup>

Neuroimaging studies in humans have implicated the vmPFC in fear extinction – extending the findings of the critical role for IL in fear extinction in rodents.<sup>13</sup> While the amygdala drives the expression of fear, the vmPFC counteracts this with the retrieval of extinction memory. Several functional magnetic resonance imaging (fMRI) studies showed that the vmPFC enhances activation while subjects retrieve the extinction memory.<sup>23–25</sup> Phelps *et al.* (2004) identified a specific region within the vmPFC, the subgenual anterior cingulate cortex ACC (sgACC, lying right beneath the corpus callosum), as more tightly tracking extinction success and skin conductance responses (SCR).<sup>24</sup> Other studies associated individual differences in extinction retrieval with the thickness of the vmPFC.<sup>26,27</sup> In a potentially important translational finding, PTSD patients were reported to retrieve extinction memory less and exhibit less vmPFC activation compared to healthy control subjects.<sup>28</sup>

GABAergic and glutamatergic signalling maintain tight control over the expression of fear conditioning and extinction.<sup>29,30</sup> Both acquisition and extinction of fear are learning processes that depend on *N*-methyl-D-aspartate (NMDA) receptor activation.<sup>31–33</sup> Fear conditioning is marked by alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors insertion in lateral nucleus synapses,<sup>34,35</sup> whereas extinction training causes the reverse.<sup>36</sup> Because of this overlap in cellular signalling, it may be challenging to

identify a compound that would selectively target one or the other for therapeutic purposes. One such compound could be the partial NMDA agonist, D-cycloserine, which was shown to enhance extinction learning (see below for further discussion).<sup>37,38</sup>

In summary, some of the salient features of the neurocircuitry of fear and fear regulation described above include the essential role of microcircuits within amygdala subnuclei, under the control of glutamate and GABA, mediating fear memory and the essential role of the vmPFC in fear extinction (Figure 9.1). The increasingly detailed model of the neural mechanisms of fear that is emerging is hoped to guide future therapeutic discovery in human anxiety disorders. With this circuitry as a foundation, below we describe specific preclinical and clinical findings in panic disorder and PTSD and highlight potential new therapeutic opportunities in these disorders.

## 9.4 Neurocircuitry of Panic Disorder

### 9.4.1 Preclinical Models

The development of valid preclinical models of panic disorder has posed challenges for research aimed at elucidating the underlying neurobiology of the disorder. The quintessential feature of panic disorder is the sudden onset of fear or dread, concurrently with a constellation of physical symptoms associated with the fight-or-flight response, which occur “out of the blue”, at least early in the course of the disorder. Simple unlearned models of anxiety in rodents such as the elevated plus maze or the open field test have proven to be powerful tools for screening putative anxiolytic compounds and characterizing neuropharmacology-behaviour relationships.<sup>39,40</sup> However, these procedures lack a certain aspect of validity as models of panic disorder and limit their usefulness in understanding the specific circuitry of the disorder and potential unique therapeutic targets.<sup>41</sup>

Models of fear and anxiety that involve learning, as described above, have yielded a wealth of information pertaining to the neurobiology of fear in animals across multiple levels of analysis, including circuits, cells, synapses, receptors and intra-cellular signalling cascades.<sup>6,17,42</sup> Current models of panic disorder posit that the anticipatory anxiety and avoidance that often develop in panic disorder may result from processes related to fear learning. According to this model, an unexpected panic attack represents a US and concurrent environmental or interoceptive stimuli come to be associated with the US through the processing of contextual fear conditioning. The challenge for animal models of panic disorder, however, has been to model the panic attack itself. Below we discuss several candidate examples of animal models and associated findings that attempt to capture these features.

One approach to the development of animal models of panic disorder has been to incorporate manipulations that have been observed to induce panic attacks in patient populations, including administering sodium lactate<sup>43–48</sup> or

the respiratory stimulant doxapram.<sup>49</sup> A model that has yielded several potentially important insights into the neurobiology of panic disorder involves first “priming” an animal by chronically inhibiting GABA synthesis in the dorsomedial hypothalamus (DMH) using L-allylglycine, a GABA synthesis inhibitor, followed by a sodium lactate challenge.<sup>43</sup> Behavioural aspects of anxiety are then measured using standard assays, including the elevated plus maze, the open-field test and social interaction, as well as physiological correlates of panic, including increased heart rate and mean arterial pressure.<sup>46</sup>

A series of experiments using this model suggest a key role for the DMH in panic-like responses and key molecular features are beginning to be elucidated. Studies have demonstrated that panic-like responses in this model can be blocked by GABA receptor A agonists infused into the DMH<sup>46</sup> and are blocked by NMDA receptor antagonists.<sup>50</sup> Using this model, anti-panic effects were demonstrated for both the benzodiazepine alprazolam and a novel glutamate metabotropic group II receptor antagonist, LY354740.<sup>44</sup> The neural pathway by which signals related to the sodium lactate challenge result in panic-like behaviours appears to involve connections between osmosensitive regions outside of the blood-brain barrier (BBB) (known as circumventricular organs (CVOs)) and the DMH with subsequent efferent projections to the amygdala and the bed nucleus of the stria terminalis (BNST) (anxious behaviours), the locus coeruleus (LC) (arousal) and parabrachial nucleus (tachypnea).<sup>46</sup> Variations on the “priming” model combined with sodium lactate infusion described above have included infusions of CRF or urocortin into the BLA.<sup>51</sup> Repetitive administration of sub-threshold doses of CRF or urocortin were found to result in priming and animals exhibited behavioural and cardiovascular responses to intravenous sodium lactate consistent with panic-like anxiety.

Pharmacological challenge with the respiratory stimulate doxapram, which triggers a panic attack in susceptible patients, in combination with established behavioural assays of anxiety may represent a useful animal model of panic for further study, although significantly less work has been conducted with this model compared to sodium lactate challenge. In a study by Sullivan *et al.* (2003), challenge with doxapram was examined in both unlearned (the open field test and the social interaction test) and learned (cue and contextual fear conditioning) rodent models of anxiety.<sup>49</sup> Doxapram increased anxiety-related behaviours in all four models and an inverted U-shaped dose-response curve was identified for fear conditioning to cue. Doxapram induced c-Fos-like immunoreactivity in the central nucleus of the amygdala but not the lateral nucleus or the nucleus tractus solitarius.

A final model of panic that we will discuss involves escape from aversive electrical stimulation of the dorsal periaqueductal gray (PAG).<sup>52</sup> It was recognized from early research that stimulation of the dorsal PAG resulted in aversive behavioural reactions in animals and that animals could learn to escape the stimulation by switching off a level. In addition, stimulation of the dorsal PAG elicits a stereotyped defence reaction in animals that mirrors ethnographic observations of the behaviour of animals in close proximity to



threat (flight, threatening postures or defensive aggression). Early research also demonstrated that similar reactions could be generated in cats by stimulating the hypothalamus or amygdala, although these reactions were more delayed and less stimulus-bound than stimulation of PAG. There are dense interconnections between the dorsal PAG, medial hypothalamus and amygdala,<sup>53</sup> which have been proposed by some to constitute a “brain defence system”.<sup>54</sup> These authors posit that brain activation shifts from rostral to caudal as a function of the defensive distance between predator and prey, a notion that has recently been granted support by the results of a virtual predator fMRI experiment in healthy humans.<sup>55</sup>

### 9.4.2 Human Behavioural and Pharmacological Challenge Studies

As noted above, patients with panic disorder are susceptible to the induction of panic-like symptoms or a full-blown panic attack following an infusion of sodium lactate (typically 0.5M sodium lactate).<sup>56–58</sup> In an early study by Liebowitz *et al.* (1985), 31 out of 43 patients with panic disorder and none of 20 normal controls developed panic-like reactions in response to infusions of sodium lactate.<sup>57</sup> Lactate-induced panic attacks were accompanied by biological changes consistent with hyperventilation and central noradrenergic activation. Of note, cortisol does not appear to increase during acute panic in this paradigm.<sup>59</sup> Despite numerous investigations, the exact mechanisms by which lactate causes panic in susceptible individuals has remained elusive. However, the preclinical study by Johnson discussed above provides support for a model by which blood pH and associated blood gas parameters changed by lactate infusion are sensed at the level of the osmosensitive regions, which is relayed to the amygdala and related fear circuitry.<sup>46</sup> Challenge with CO<sub>2</sub> inhalation can also stimulate panic in susceptible individuals, although less reliably than with sodium lactate challenge, potentially through a similar mechanism.<sup>60–62</sup>

Challenge studies with the alpha-2 adrenergic receptor antagonist yohimbine have provided substantial support to the hypothesis of abnormal central noradrenergic functioning in patients with panic disorder.<sup>63–68</sup> Yohimbine is known to increase the firing rate of noradrenergic neurons in the LC *via* blockage of the alpha-2 adrenergic auto-receptor through inhibition of negative feedback. In an initial study, Charney *et al.* (1984) administered yohimbine to 20 healthy subjects and 39 patients with agoraphobia and panic attacks and found significantly greater increases in anxiety, nervousness, palpitations, hot and cold flashes, restlessness, tremors, piloerection and sitting systolic BP in panic patients compared with healthy subjects.<sup>63</sup> There were significant correlations between the yohimbine-induced rise in plasma levels of the nor-epinephrine (NE) metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) and patient-rated anxiety and nervousness and the frequency of reported panic attacks. In a larger follow-up study, yohimbine produced panic attacks in 37 of 68 patients with panic disorder and 1 of 20 healthy control participants.<sup>67</sup>



The patients reporting yohimbine-induced panic attacks had significantly larger increases in plasma MHPG, cortisol, systolic blood pressure and heart rate than the healthy subjects. Taken together, these findings support the hypothesis relating high noradrenergic neuronal activity to the pathophysiology of panic attacks, but only in a subgroup of patients (Figure 9.1).

### 9.4.3 Human Neuroimaging Studies

#### 9.4.3.1 Functional Neuroimaging

A series of early functional neuroimaging studies in panic disorder measured either regional cerebral blood flow (rCBF) or regional cerebral glucose metabolic rate (rCMRgluc) in patients with panic disorder utilizing a pharmacological challenge to induce panic. An early single-photon emission computed tomography (SPECT) study with inhaled xenon-133 by Stewart *et al.* (1988) found that rCBF increased in control subjects and in panic disorder patients who did not panic following challenge with sodium lactate.<sup>69</sup> In contrast, patients who did panic had evidence of a reduced increase or even a decrease in rCBF compared to baseline. This result may be consistent with the fact that hyperventilation that often accompanies a panic attack can result in a reflex vasoconstriction of cerebral arteries, which could produce reductions in CBF that are physiological but not neural in origin. A second study of rCBF using positron emission tomography (PET) found that lactate-induced panic was associated with significant blood flow increases in a number of brain regions, including the temporal poles, insular cortex, putamen and brainstem specifically in panicking patients with panic disorder but not in non-panicking patients or in healthy controls.<sup>70</sup> In a third study, Woods *et al.* (1988) employed yohimbine challenge with SPECT in panic patients and observed bilateral reductions in rCBF in the frontal cortex in association with increased anxiety in patients but not in controls.<sup>71</sup>

Studies conducted in patients with panic disorder under unprovoked/non-panic conditions have also yielded conflicting results, reporting both decreased and increased rCBF or rCMRgluc compared to healthy controls.<sup>72–75</sup> A more recent resting [18F]-fludeoxyglucose (FDG) PET study found elevated rCMRgluc within the amygdala, hippocampal regions, brainstem and cerebellum in 12 panic patients compared to 22 matched control subjects.<sup>76</sup> A follow-up study by the same group reported decreased rCMRgluc in the right hippocampus, left anterior cingulate, left cerebellum and brainstem, and concomitant increased rCMRgluc in medial frontal structures in panic patients following a successful course of CBT.<sup>77</sup>

An initial fMRI study in six patients with panic disorder and six matched health controls using a block design with imagery of neutral, moderate and high anxiety situations showed increased blood oxygenation level-dependent (BOLD) signal to high *versus* low anxiety imagery in inferior frontal cortex, anterior and posterior cingulate and hippocampus in panic compared to control participants.<sup>78</sup> Partially congruent with these results, another small fMRI

study of threat or neutral words showed greater threat-related activation in the left posterior cingulate and left middle frontal cortices in panic patients compared to healthy volunteers.<sup>79</sup> Several additional fMRI studies in panic disorder have been conducted and have yielded somewhat inconsistent findings, reporting both decreased and increased cortical and amygdala activation patterns in response to emotional stimuli in panic disorder compared to healthy controls.<sup>80–82</sup> Interestingly, a recent fMRI study found that patients with panic disorder, compared to both healthy controls and patients with PTSD, evidenced attenuated BOLD responses to threat compared to safe cues in multiple brain regions, including ACC, striatum, amygdala and brainstem.<sup>83</sup>

Two studies using an imaging genetics paradigm investigated the influence of the val158met polymorphism in COMT and two polymorphisms in the serotonin system, respectively, on BOLD responses in panic disorder.<sup>84,85</sup> In the first study, the authors found tentative support for a relationship between a polymorphism in the gene coding for the 5-HT1A receptor (-1019C/G) and BOLD response in prefrontal cortical regions and amygdala to fearful or happy faces.<sup>85</sup> In the second study, carriers of at least one COMT risk allele (472G) evidenced heightened right amygdala and left orbital frontal cortex (OFC) to fearful faces.<sup>84</sup> These studies provide provisional evidence that genetic polymorphisms may be linked to specific neural circuitry abnormalities in panic disorder and specifically further implicates prefrontal cortical structures and amygdala in the disorder.

#### 9.4.3.2 Structural MRI

Several quantitative structural MRI studies have found evidence for reduced volume of temporal lobe structures in panic disorder, including the amygdala and hippocampus.<sup>86–89</sup> In contrast to these findings, Protopopescu *et al.* (2006) found evidence for increased volume of brainstem structures and trend toward increased volume of temporal lobe structures.<sup>90</sup> A study by Uchida *et al.* (2008) found evidence of increased brainstem, parahippocampal gyrus and insula, together with relatively decreased ACC volume.<sup>91</sup> Another recent study found evidence for increased midbrain volume in PD, including the PAG, which correlated with behavioural measures of anxiety.<sup>92</sup> The volumetric MRI studies in panic disorder conducted to date exhibit discrepancies in directionality of findings in some cases, although taken together support a neurocircuitry model of panic disorder that includes brainstem, hippocampus/parahippocampus, amygdala and vmPFC/ACC.

#### 9.4.3.3 Neurochemical Imaging

Several early studies using SPECT and [<sup>123</sup>I]-iomazenil uptake demonstrated regional alterations in GABA receptor binding in patients with panic disorder.<sup>93–95</sup> For example, Kascka *et al.* compared a group of 9 patients with panic disorder and depression with a matched control group of 9 dysthymic

patients without panic disorder and found bilateral decreases in uptake of [123I]-iomazenil in the inferior frontal and temporal lobes in the disorder group relative to the comparison group.<sup>94</sup> Using optimized methods, Bremner *et al.* found decreased binding to the GABA receptor benzodiazepine site in left hippocampus and precuneus in panic disorder patients relative to healthy controls.<sup>96</sup>

In addition to SPECT, several groups have investigated the GABA system in panic using [11C]-flumazenil PET and have documented reduced binding in ventral and dorsal prefrontal cortex (PFC), ACC and insula and temporal cortex in panic disorder relative to healthy controls.<sup>97–99</sup> Unexpectedly, one study found increased GABA receptor binding in panic compared to controls in the hippocampus/parahippocampal gyrus bilaterally and the left dorsolateral PFC (DLPFC).<sup>99</sup> In this study, the severity of panic and anxiety symptoms correlated positively with GABA receptor binding in the PFC but negatively with binding in the hippocampus/parahippocampal gyrus.

Neumeister and colleagues investigated the 5-HT<sub>1A</sub> receptor in panic disorder using [18F]-FCWAY PET and found receptor distribution was lower in the PD group relative to the control group in the ACC, posterior cingulate cortex (PCC), and in brainstem raphe nuclei.<sup>100</sup> Using proton magnetic resonance spectroscopy (1H-MRS), GABA concentration was estimated to be reduced in the occipital cortex in panic disorder.<sup>101</sup> However, in a more recent 1H-MRS study by Hasler *et al.*, the authors examined 17 panic patients and 17 matched controls and found no difference in GABA concentration in PFC.<sup>102</sup> In addition, there was no significant difference in glutamate/glutamine (Glx), choline, or *N*-acetyl aspartate concentrations. Of note, the region of interest (ROI) in the previous studies was the occipital cortex, while the Hasler *et al.* report examined a region of the PFC.

#### 9.4.4 Genetics Studies

Initial evidence for a genetic component to panic disorder comes from family and twin studies, which document a heritability between 0.28 and 0.43.<sup>103,104</sup> A series of genetic association studies using a candidate gene approach have found evidence for association with panic disorder of several genes, including COMT,<sup>105,106</sup> MAOA,<sup>107</sup> HTR2A,<sup>108,109</sup> TPH2,<sup>110–112</sup> SLC6A4<sup>113,114</sup> and ADORA2A.<sup>115</sup> Of note, the majority of these findings await replication, or contradictory reports have been published.<sup>116–119</sup> Using genomic linkage analysis, a region on chromosome 15 near two genes coding for subunits of the GABA<sub>A</sub> receptor (GABRB3, GABRA5) was identified as a susceptibility locus.<sup>120</sup> This finding, however, awaits replication. See Maron *et al.* (2010) for a comprehensive review of candidate gene association and linkage studies.<sup>121</sup> Genome-wide association studies (GWASs) have only recently begun to be conducted in panic disorder and initial findings await replication.<sup>122–124</sup> A case-control candidate gene association study of several 5-HT-related genes

(HTR1A, HTR2A and the 5-HTTLPR) in PD found evidence for association with HTR1A, but not the other genes examined.<sup>125</sup>

A recent genome-wide scan study using microsatellite markers in panic disorder and control individuals from an isolated population found evidence for the amiloride-sensitive cation channel 1 (ACCN1) located on chromosome 17q11.2-q12 as a potential candidate gene. However, a second analysis in an independent case-control sample failed to provide evidence for an association between ACCN1 and panic disorder.<sup>126</sup> A recent study analyzed the Ile408Val polymorphism in the hypocretin receptor 1 (HCRTR1) gene and the Val308Iso (G1246A) polymorphism in the hypocretin receptor 2 (HCRTR2) gene in a sample of 215 panic disorder patients and 454 controls.<sup>127</sup> In this study, the Iso allele of the HCRTR2 polymorphism was significantly more frequent in patients than in controls, although there was no difference between cases and controls in the frequency of the polymorphism in the HCRTR1. A novel case-control study of single-nucleotide polymorphisms (SNPs) tagging microRNAs (miRNAs), implicated in neuronal differentiation and synaptic plasticity, found evidence for association of several miRNAs with panic disorder.<sup>128</sup> The authors report that the associated miRNAs function to repress several candidate genes for PD, including GABRA6, CCKBR, POMC, BDNF, HTR2C, MAOA and RGS2. While intriguing, these findings await replication.

## 9.5 Neurocircuitry of PTSD

### 9.5.1 Preclinical Models

Among the different anxiety disorders, PTSD may be most directly related to abnormalities in fear learning and in particular to a failure to extinguish learned fear associations.<sup>14,129</sup> In this view, the traumatic experience that leads to PTSD involves associative learning between a primary aversive stimulus concomitant with fear and pain (the traumatic stress itself – the US) and a myriad of internal and external cues that subsequently come to be associated with the experience of fear (*e.g.* these cues then coming to represent CS stimuli). This process produces the cardinal symptoms of PTSD: intrusive memories of the trauma (often triggered by environmental cues that no longer signify danger), avoidance of reminders of the trauma (to prevent the fear response) and hyperarousal (chronic heightened fear state). It is important to note that animal models of fear learning generally allow for the study of mechanisms of normal processes, which may provide insight into particular aspects of PTSD or other anxiety disorders. The development of a valid animal model of PTSD *per se* has proven to be a challenge for the field, but several models have been partially validated. Below we briefly review the single prolonged stress (SPS) model of PTSD. See Yamamoto *et al.* (2009) for a thorough recent review of animal models of PTSD.<sup>130</sup>

In the SPS model, animals are exposed to restraint and forced-swim stress followed by anaesthesia with ether.<sup>131,132</sup> Importantly for the potential face and

construct validity of this model, SPS rats have been reported to manifest enhanced acquisition of fear conditioning<sup>133</sup> and deficient fear extinction.<sup>134</sup> Regarding neurobiology, hypothalamic-pituitary-adrenal (HPA) axis function in particular has been investigated using the SPS model and findings suggest enhanced negative feedback of the HPA, consistent with human investigations in PTSD.<sup>135–137</sup> Glutamatergic function and the NMDA receptor have also been studied using this model; NMDA receptors in the hippocampus have been found to be both reduced<sup>138</sup> and elevated<sup>134</sup> following the SPS procedure. Hippocampal GABA levels were also found to be reduced,<sup>138</sup> consistent with a radioligand PET study in human PTSD (see below).<sup>139</sup>

## 9.5.2 Human Behavioural and Pharmacological Challenge Studies

### 9.5.2.1 *Studies of the HPA Axis*

Hypotheses related to HPA axis dysfunction in PTSD have gained support from a variety of empirical studies, although a clear picture of HPA axis dysfunction in PTSD has remained elusive. For example, studies of cortisol levels in patients with PTSD compared to control participants have yielded inconsistent findings.<sup>140</sup> One of the most common endocrinological findings in PTSD is exaggerated suppression of the HPA axis to low-dose dexamethasone.<sup>135–137</sup> An initial study of the HPA axis in PTSD using a low dose of dexamethasone found that PTSD patients showed greater suppression of cortisol in response to dexamethasone than did healthy control subjects.<sup>135</sup> In a follow-up study, Yehuda *et al.* (2006) measured ACTH and cortisol at baseline and in response to dexamethasone in subjects with and without PTSD and found greater suppression of ACTH and cortisol in PTSD in response to dexamethasone.<sup>137</sup> Interestingly, parental PTSD appears to influence an individual's response to HPA challenge.<sup>141,142</sup>

### 9.5.2.2 *Studies of the Noradrenergic System*

A series of studies in patients with PTSD have demonstrated noradrenergic abnormalities in PTSD. An initial challenge study with yohimbine documented significant increases in PTSD symptoms of intrusive traumatic thoughts and emotional numbing, as well as panic attacks in some patients.<sup>143</sup> Patients with PTSD also had larger yohimbine-induced increases in plasma MHPG levels, sitting systolic blood pressure and heart rate than those in healthy subjects. In a follow-up study, PTSD patients underwent challenge with either yohimbine or meta-chlorophenylpiperazine (*m*-CPP).<sup>144</sup> In this study, a subgroup of PTSD patients experienced yohimbine-induced panic attacks and had significantly greater increases compared with controls in anxiety, panic and PTSD symptoms while a different subgroup experienced *m*-CPP-induced panic attacks and had significantly greater increases compared with controls in anxiety, panic and

PTSD symptoms, and in standing diastolic blood pressure. In a study that directly measured cerebrospinal fluid (CSF) NE levels, NE concentrations were significantly higher in the men with PTSD than in the healthy men.<sup>145</sup> Moreover, CSF NE levels strongly and positively correlated with the severity of PTSD symptoms.

### 9.5.2.3 *Studies of Neuropeptides*

In a study investigating neuropeptide Y (NPY) in PTSD and response to yohimbine challenge, PTSD subjects had lower baseline plasma NPY and blunted yohimbine-stimulated increases in plasma NPY compared with the healthy control subjects.<sup>146</sup> Within the PTSD group, baseline plasma NPY levels correlated negatively with combat exposure scale scores, baseline PTSD and panic symptoms, and yohimbine-stimulated increases in MHPG and systolic blood pressure. In a second study of NPY in PTSD, plasma NPY was measured in 11 non-exposed veterans, 11 combat-exposed veterans without post-traumatic stress disorder (PTSD) and 12 veterans with current PTSD.<sup>147</sup> In this study, higher NPY levels were found in exposed veterans without PTSD than in non-exposed but comparable levels were found in veterans with current PTSD. In a more recent study, NPY concentrations in CSF from 10 male subjects with chronic combat-related PTSD and from 13 healthy men revealed PTSD patients had significantly lower concentrations of CSF NPY.<sup>148</sup>

A study examining the neuropeptide substance P in PTSD found that CSF concentrations of the peptide were significantly elevated at baseline compared to healthy control subjects.<sup>149</sup> The study further found that CSF substance P significantly increased in PTSD patients in response to a traumatic videotape challenge. A recent randomized clinical trial of a substance P receptor antagonist in PTSD, however, was negative.<sup>150</sup> A recent study of the hypothalamic neuropeptide orexin-A found reduced levels of the peptide in the CSF of patients with PTSD.<sup>151</sup> In addition, CSF orexin-A concentrations negatively correlated with PTSD symptom severity. Neuropeptides may represent an important new target for therapeutic development in PTSD and other anxiety disorders; however, further research will be needed before more definitive conclusions can be drawn.

## 9.5.3 Human Neuroimaging Studies

### 9.5.3.1 *Functional Neuroimaging*

A convergence of functional imaging studies in PTSD suggests baseline overactivity of the amygdala and/or heightened reactivity of the amygdala to threat or fear-related stimuli concomitantly with reduced baseline activity or reduced responsiveness in medial PFC (mPFC) regions.<sup>28,129,152–157</sup> Several early PET studies found increased glucose metabolism or blood flow in the amygdala at rest or in response to trauma-related stimuli.<sup>158–161</sup> Using fMRI, BOLD



responses in the amygdala to fearful faces or other threat-related stimuli were found to be exaggerated in PTSD.<sup>152,162</sup> It should be noted, however, that several studies have failed to document exaggerated amygdala activity.<sup>163,164</sup> Interestingly, higher pre-treatment amygdala and ventral ACC activity was found to predict response to CBT in an fMRI study utilizing masked fearful faces.<sup>165</sup>

Studies reporting reduced mPFC activity in PTSD have included provocation studies with trauma-related stimuli,<sup>160,163,166</sup> as well as studies conducted with non-trauma related emotional stimuli<sup>162,167,168</sup> and non-emotional cognitive studies.<sup>169</sup> In a novel study combining rCBF PET measures with blood endocrine changes in response to symptom provocation, Liberzon *et al.* (2007) found that changes in ACTH covaried with rCBF specifically within mPFC and ACC regions, as well as in the insula in PTSD.<sup>170</sup>

A quantitative meta-analysis by Etkin and Wager (2007) compared fMRI and PET studies of PTSD, social phobia, specific phobia and fear conditioning in healthy individuals and found that only patients with PTSD demonstrated regions of hypoactivation compared to healthy controls.<sup>156</sup> These regions included the OFC, regions of mPFC [dmPFC, vmPFC, rostral (rACC)], mid-cingulate, anterior hippocampus and parahippocampal gyrus. In all studies of PTSD included in that review, mPFC activity negatively correlated with symptom severity. Taken together, the existent literature on mPFC function in PTSD may suggest a unique role for this structure in the pathophysiology of the disease.

### 9.5.3.2 Structural MRI

Several neuroimaging studies of the hippocampus have found decreased hippocampal volumes in PTSD compared to non-PTSD groups.<sup>171–173</sup> Studies have also found decreased hippocampal function either at rest or in response to a cognitive or emotional challenge.<sup>163,173,174</sup> However, discrepant finds have also been reported.<sup>175–178</sup> Several studies have investigated potential volumetric alterations of the ACC in PTSD.<sup>179,180</sup> An initial study found that PTSD was associated with smaller ACC volumes<sup>179</sup> and a follow-up study by the same group reported that the COMT Val158Met genotype interacted with PTSD diagnosis to predict smaller ACC volume.<sup>180</sup> The difference in ACC volume between the participants without PTSD and participants with PTSD was greater among individuals homozygous for the Val allele than among carriers of the Met allele.

### 9.5.3.3 Neurochemical Imaging

An early *in vivo* study of the GABA system in PTSD found reduced binding in the PFC in PTSD compared to healthy volunteers using SPECT imaging of [123I]-iomazenil.<sup>181</sup> However, a second SPECT study using the same tracer failed to replicate these findings.<sup>182</sup> A more recent study utilizing the selective



PET radiotracer [11C]-flumazenil found evidence for widespread decreases in cortical and subcortical GABA receptors in PTSD.<sup>139</sup> A PET study utilizing [18F]-FCWAY to investigate the 5-HT<sub>1A</sub> receptor found no regional receptor binding differences between PTSD and healthy control groups.<sup>183</sup> In a study of the mu-opioid system using the selective radiotracer [11C]-carfentanil, Liberzon *et al.* (2007) documented reduced mu-opioid receptor binding in the amygdala, nucleus accumbens and insula and increased binding in the OFC in both PTSD and trauma-exposed non-PTSD samples. PTSD was specifically associated with reduced receptor binding in the ACC.<sup>184</sup>

Recent studies have begun to investigate the 5-HT<sub>1B</sub> receptor and the 5-HT transporter (5-HTT) in PTSD.<sup>185,186</sup> In a large PET study, 96 individuals in 3 study groups (PTSD (n=49), trauma-control (n=20) and healthy-control (n=27)) underwent PET scanning using the recently developed 5-HT<sub>1B</sub> receptor-selective radiotracer [11C]-P943.<sup>185</sup> In this study it was found that a history of severe trauma exposure was associated with marked reductions in [11C]-P943 binding potential in the caudate, the amygdala and the ACC and that participant age at first trauma exposure was strongly associated with low [11C]-P943 binding potential. Developmentally earlier trauma exposure also was associated with greater PTSD symptom severity and major depression co-morbidity. In a second study, 5-HTT levels were assessed *in vivo* in patients with PTSD using the recently developed 5-HTT-selective radiotracer [11C]-AFM.<sup>186</sup> Here it was found that [11C]-AFM binding potential was significantly reduced in the amygdala in patients with PTSD compared to healthy control participants and the level of reduction was correlated with greater severity of depressive and anxiety symptoms at the time of scanning. These studies clearly implicate the 5-HT system in PTSD, although the specific nature of the dysfunction and mechanisms of pathogenicity remain to be fully characterized.

### 9.5.4 Genetics Studies

Family and twin studies suggest that vulnerability to PTSD is moderately heritable (30–40%).<sup>187,188</sup> Linkage and association studies in PTSD, however, face special methodological difficulties stemming from the requirement of environmental trauma exposure in the etiology of the disorder. Conversely, PTSD may uniquely lend itself to studies of gene x environment interactions.<sup>189</sup> Several candidate gene studies have reported that the short allele of the 5-HT length repeat polymorphism (5-HTTLRP) increases the vulnerability to develop PTSD,<sup>190–193</sup> and may predict poor treatment outcome.<sup>194</sup> In one of the larger studies to date, Xie *et al.* (2009) sought to examine the effects of childhood adversity, adult traumata, 5-HTTLPR genotypes and gene x environment interactions on PTSD in a cross-sectional candidate gene study.<sup>192</sup> Similar to other studies, the authors found that 5-HTTLPR genotype alone did not predict PTSD but that it interacted with adult traumatic events and childhood adversity to increase the risk for PTSD.

Using a similar cross-sectional candidate gene approach, Binder *et al.* (2008) examined genetic polymorphisms at the glucocorticoid receptor (GR)-related gene *FKBP5* and interactions with childhood trauma in the development of PTSD.<sup>195</sup> The authors found that several SNPs associated with *FKBP5* interacted with child abuse to predict level of adult PTSD symptoms. Of note, no SNP directly predicted PTSD symptoms, consistent with findings from studies of the 5-HTTLRP. A recent study examined associations between SNPs within genes coding for the pituitary adenylate cyclase-activating polypeptide (PACAP) and the PACAP receptor (PAC1) and PTSD and found evidence of a sex-specific association in females.<sup>196</sup> Blood levels of PACAP were also correlated with measurements of fear and PTSD symptoms in females. This neuropeptide, widely implicated in cellular responses to stress, has not been described in human anxiety disorder previously and may suggest a new avenue for therapeutic and translational research in PTSD or other anxiety disorders.<sup>196</sup>

## 9.6 Examples of Novel Therapeutic Targets in Anxiety Disorders

### 9.6.1 Glutamate and GABA

As described above in the section on neurocircuitry of fear, the microcircuitry controlling fear learning within the amygdala is tightly regulated by glutamatergic and GABAergic signalling. Therefore, therapeutic research targeting these systems may hold promise for novel therapeutic development for anxiety disorders. Insufficient top-down control from the vmPFC to the amygdala hypothesized in PTSD suggests involvement of glutamatergic pathways in PTSD. NMDA receptor antagonists are known to interfere with the expression of anxiety-related behaviour in animals, consistent with the involvement of NMDA receptors in memory consolidation processes.<sup>197</sup> Of note, McGhee *et al.* (2008) found that in a group of burned servicemen those treated with the NMDA receptor antagonist ketamine during hospitalization had lower incidence of developing PTSD.<sup>198</sup> D-cycloserine, the partial NMDA agonist described above, has been shown to enhance extinction learning in animals and several clinical studies indicate therapeutic potential in anxiety disorders.<sup>37,38</sup> The expansion of the identification and characterization of ligands specific for metabotropic glutamate receptors (mGluRs) further enhances our potential ability to modulate the glutamate system as a novel therapeutic approach in anxiety disorders.<sup>199</sup>

In addition to disturbances in glutamate signalling, disruptions in signalling within GABAergic interneurons in the amygdala may contribute to abnormal fear learning in anxiety disorders and may provide a novel treatment target. A pharmacological challenge anxiety model was recently used to demonstrate anti-anxiety effects of a novel translocator protein (18 kD) ligand, XBD173.<sup>200</sup> The authors found that XBD173 enhances GABA transmission indirectly

through the generation of GABAergic neurosteroids. Ring A-reduced neurosteroids are endogenous metabolites of the hormone progesterone and are known to be positive allosteric modulators of type A GABA receptors and exert anxiolytic effects in animal models. The authors also found that XBD173 exerted anti-panic activity in healthy male volunteers using the CCK4 challenge. These encouraging initial results suggest that translocator protein ligands may represent new candidates for fast-acting anxiolytic drugs with less severe side-effects than benzodiazepines.

### 9.6.2 Neuropeptides

Neuropeptide Y signalling is known to modulate stress responses in animals and a series of studies in humans supports the hypothesis that NPY may represent a protective factor in the face of stress.<sup>201,202</sup> NPY produces anxiolytic effects in animal models and enhances the extinction of conditioned fear at the level of the basolateral amygdala.<sup>203</sup> Further, repeated administration of NPY directly into the basolateral nucleus resulted in resilient behavioural responses in an acute restraint paradigm as measured in the social interaction test.<sup>204</sup> These studies suggesting the potential protective role for NPY under conditions of high stress are consistent with recent human genetics studies implicating variations in the NPY gene in emotion and the stress response.<sup>205,206</sup>

A recent preclinical study utilizing a variation of the priming models discussed above suggests a key role for the neuropeptide orexin in panic-related behaviours elicited using the model described above.<sup>48</sup> Orexin-containing neurons are specifically localized to the DMH and lateral hypothalamus (LH) and orexin is known to regulate key aspects of arousal, emotion, sleep and appetite.<sup>207</sup> The authors report that orexin-containing cells within the DMH are specifically activated by sodium lactate challenge in panic-prone animals and that activation within these cells correlated with anxiety-related behaviours. Further, blocking transcription of the gene coding orexin or administering an orexin-1 receptor antagonist abolished the panic behaviour. This study suggests a potential new target in the orexin system for drug development for panic disorder. In further support of this hypothesis, there is recent human genetic evidence of involvement of the orexin system in panic disorder, as reviewed above.<sup>127</sup>

Very recently a completely novel neuropeptide, PACAP, has been highlighted as potentially involved in PTSD (see discussion above).<sup>196</sup> In a series of clinical and preclinical experiments, the authors showed that SNPs within the PACAP gene were associated with PTSD in females and that methylation of the gene in peripheral blood was also associated with PTSD in females. While the role of PACAP in normal physiology and its potential role in the pathophysiology of PTSD remains uncertain, future studies will continue to investigate the role of this system in anxiety disorders and the potential to target this system in future treatment development.

## 9.7 Summary and Conclusion

Anxiety disorders are among the most common and disabling of psychiatric conditions. Although current treatment strategies are not optimally effective for many patients, ongoing basic, clinical and translational research aimed at understanding the neurobiology underlying the disorders is shedding new light on mechanisms of disease and opening up potential new avenues for therapeutic discovery. In this chapter we described advances in our understanding of basic neurocircuitry of fear emerging from work in animals utilizing models of fear conditioning. This work has highlighted microcircuitry within subnuclei of the amygdala underlying fear learning and extinction and the tight control over these processes by glutamatergic inputs from medial PFC structures and elsewhere. The balance between glutamatergic and GABAergic signalling in these subnuclei appears critical for normal fear learning and extinction. Human neuroimaging research in healthy populations and in patients with anxiety disorders supports a translational model of fear involving the regulation of the amygdala and related structures by medial aspects of PFC and an imbalance between these structures in anxiety disorders. Neuroendocrine and neurotransmitter findings in clinical populations, as well as findings from basic neuropharmacology, are pointing towards potential novel treatment avenues for these disorders. We highlighted example targets from the glutamatergic, GABAergic and neuropeptide. As is often the case with neuropharmacology, the challenge will be to develop small molecules with a high degree of selectivity to allow for the precise targeting of cell subtypes while limiting undesirable side-effects. Future research will be necessary to establish the efficacy of any potential new therapeutic candidate from these disabling conditions.

## Conflict of Interest Disclosure

Dr Charney has been named as an inventor on a use-patent of ketamine for the treatment of depression. If ketamine were shown to be effective in the treatment of depression and received approval from the Food and Drug Administration for this indication, Dr Charney and the Mount Sinai School of Medicine could benefit financially. All other authors report no competing interests.

## Acknowledgements

Dr Murrough is supported by a Career Award grant from NIH/NIMH (grant number 1K23MH094707-01) and receives additional research support from the Brain and Behavior Research Foundation (NARSAD Young Investigator Award) and the American Foundation for Suicide Prevention. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIMH, NIH or other funding agency. The authors would also like to acknowledge the generous support of Mount Sinai School of Medicine and the Mount Sinai Friedman Brain Institute.

## References

1. R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Merikangas and E. E. Walters, *Arch. Gen. Psychiatry*, 2005, **62**, 593–602.
2. American Psychiatric Association. Task Force on DSM-IV, *Diagnostic and statistical manual of mental disorders: DSM-IV-TR*, American Psychiatric Association, Washington, DC; New York, 2000.
3. W. J. Katon, *N. Engl. J. Med.*, 2006, **354**, 2360–2367.
4. J. W. Murrough, S. J. Mathew and D. S. Charney, in *Clinical Psychiatry Essentials*, ed. L. W. Roberts, Lippincott Williams and Wilkins, Philadelphia, 2010, pp. 207–224.
5. E. A. Phelps and J. E. LeDoux, *Neuron*, 2005, **48**, 175–187.
6. J. E. LeDoux, *Annu. Rev. Neurosci.*, 2000, **23**, 155–184.
7. J. C. Repa, J. Muller, J. Apergis, T. M. Desrochers, Y. Zhou and J. E. LeDoux, *Nat. Neurosci.*, 2001, **4**, 724–731.
8. B. S. Kapp, P. J. Whalen, W. F. Supple and J. P. Pascoe, in *The Amygdala: Neurobiological Aspects of Emotion, Memory and Mental Dysfunction*, ed. J. P. Aggleton, Wiley-Liss, New York, 1992, pp. 229–254.
9. G. P. McNally, J. P. Johansen and H. T. Blair, *Trends Neurosci.*, 2011, **34**, 283–292.
10. S. Ciochi, C. Herry, F. Grenier, S. B. Wolff, J. J. Letzkus, I. Vlachos, I. Ehrlich, R. Sprengel, K. Deisseroth, M. B. Stadler, C. Muller and A. Luthi, *Nature*, 2010, **468**, 277–282.
11. E. Likhtik, D. Popa, J. Apergis-Schoute, G. A. Fidacaro and D. Pare, *Nature*, 2008, **454**, 642–645.
12. G. J. Quirk, R. Garcia and F. Gonzalez-Lima, *Biol. Psychiatry*, 2006, **60**, 337–343.
13. M. R. Milad, S. L. Rauch, R. K. Pitman and G. J. Quirk, *Biol. Psychol.*, 2006, **73**, 61–71.
14. S. L. Rauch, L. M. Shin and E. A. Phelps, *Biol. Psychiatry*, 2006, **60**, 376–382.
15. A. Amir, T. Amano and D. Pare, *J. Neurophysiol.*, 2011, **105**, 3054–3066.
16. M. R. Milad and G. J. Quirk, *Nature*, 2002, **420**, 70–74.
17. G. J. Quirk and D. Mueller, *Neuropsychopharmacology*, 2008, **33**, 56–72.
18. F. Sotres-Bayon, C. K. Cain and J. E. LeDoux, *Biol. Psychiatry*, 2006, **60**, 329–336.
19. C. Herry, F. Ferraguti, N. Singewald, J. J. Letzkus, I. Ehrlich and A. Luthi, *Eur. J. Neurosci.*, 2010, **31**, 599–612.
20. I. Ehrlich, Y. Humeau, F. Grenier, S. Ciochi, C. Herry and A. Luthi, *Neuron*, 2009, **62**, 757–771.
21. D. Sierra-Mercado, N. Padilla-Coreano and G. J. Quirk, *Neuropsychopharmacology*, 2011, **36**, 529–538.
22. M. E. Bouton, *Psychol. Bull.*, 1993, **114**, 80–99.
23. M. R. Milad, C. I. Wright, S. P. Orr, R. K. Pitman, G. J. Quirk and S. L. Rauch, *Biol. Psychiatry*, 2007, **62**, 446–454.

24. E. A. Phelps, M. R. Delgado, K. I. Nearing and J. E. LeDoux, *Neuron*, 2004, **43**, 897–905.
25. R. Kalisch, E. Korenfeld, K. E. Stephan, N. Weiskopf, B. Seymour and R. J. Dolan, *J. Neurosci.*, 2006, **26**, 9503–9511.
26. M. R. Milad, B. T. Quinn, R. K. Pitman, S. P. Orr, B. Fischl and S. L. Rauch, *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 10706–10711.
27. C. A. Hartley, B. Fischl and E. A. Phelps, *Cereb. Cortex*, 2011, **21**, 1954–1962.
28. M. R. Milad, R. K. Pitman, C. B. Ellis, A. L. Gold, L. M. Shin, N. B. Lasko, M. A. Zeidan, K. Handwerker, S. P. Orr and S. L. Rauch, *Biol. Psychiatry*, 2009, **66**, 1075–1082.
29. A. L. Mahan and K. J. Ressler, *Trends Neurosci.*, 2012, **35**(1), 24.
30. H. C. Pape and D. Pare, *Physiol. Rev.*, 2010, **90**, 419–463.
31. J. L. Brigman, T. Wright, G. Talani, S. Prasad-Mulcare, S. Jinde, G. K. Seabold, P. Mathur, M. I. Davis, R. Bock, R. M. Gustin, R. J. Colbran, V. A. Alvarez, K. Nakazawa, E. Delpire, D. M. Lovinger and A. Holmes, *J. Neurosci.*, 2010, **30**, 4590–4600.
32. J. M. Zimmerman and S. Maren, *Eur. J. Neurosci.*, 2010, **31**, 1664–1670.
33. W. A. Falls, M. J. Miserendino and M. Davis, *J. Neurosci.*, 1992, **12**, 854–863.
34. M. Mokin, Z. Zheng and J. Keifer, *J. Neurophysiol.*, 2007, **98**, 1278–1286.
35. S. Rumpel, J. LeDoux, A. Zador and R. Malinow, *Science*, 2005, **308**, 83–88.
36. R. L. Clem and R. L. Haganir, *Science*, 2010, **330**, 1108–1112.
37. A. J. Guastella, P. F. Lovibond, M. R. Dadds, P. Mitchell and R. Richardson, *Behav. Res. Ther.*, 2007, **45**, 663–672.
38. K. J. Ressler, B. O. Rothbaum, L. Tannenbaum, P. Anderson, K. Graap, E. Zimand, L. Hodges and M. Davis, *Arch. Gen. Psychiatry*, 2004, **61**, 1136–1144.
39. S. Pellow and S. E. File, *Pharmacol. Biochem. Behav.*, 1986, **24**, 525–529.
40. L. Prut and C. Belzung, *Eur. J. Pharmacol.*, 2003, **463**, 3–33.
41. W. T. McKinney, *Semin. Clin. Neuropsychiatry*, 2001, **6**, 68–78.
42. M. R. Delgado, K. I. Nearing, J. E. LeDoux and E. A. Phelps, *Neuron*, 2008, **59**, 829–838.
43. A. Shekhar, S. R. Keim, J. R. Simon and W. J. McBride, *Pharmacol. Biochem. Behav.*, 1996, **55**, 249–256.
44. A. Shekhar and S. R. Keim, *Neuropharmacology*, 2000, **39**, 1139–1146.
45. T. J. Sajdyk, S. R. Keim, S. R. Thielen, S. D. Fitz and A. Shekhar, *Curr. Protoc. Neurosci.*, 2003, Chapter 9, Unit 9.17.
46. P. L. Johnson, W. A. Truitt, S. D. Fitz, C. A. Lowry and A. Shekhar, *Neuropsychopharmacology*, 2008, **33**, 2093–2107.
47. A. I. Molosh, P. L. Johnson, S. D. Fitz, J. A. Dimicco, J. P. Herman and A. Shekhar, *Neuropsychopharmacology*, 2010, **35**, 1333–1347.
48. P. L. Johnson, W. Truitt, S. D. Fitz, P. E. Minick, A. Dietrich, S. Sanghani, L. Traskman-Bendz, A. W. Goddard, L. Brundin and A. Shekhar, *Nat. Med.*, 2010, **16**, 111–115.



49. G. M. Sullivan, J. Apergis, J. M. Gorman and J. E. LeDoux, *Biol. Psychiatry*, 2003, **53**, 863–870.
50. P. L. Johnson and A. Shekhar, *J. Neurosci.*, 2006, **26**, 7093–7104.
51. T. J. Sajdyk, D. A. Schober, D. R. Gehlert and A. Shekhar, *Behav. Brain Res.*, 1999, **100**, 207–215.
52. F. G. Graeff, *Neurosci. Biobehav. Rev.*, 2004, **28**, 239–259.
53. N. S. Canteras, E. R. Ribeiro-Barbosa and E. Comoli, *Neurosci. Biobehav. Rev.*, 2001, **25**, 661–668.
54. N. McNaughton and P. J. Corr, *Neurosci. Biobehav. Rev.*, 2004, **28**, 285–305.
55. D. Mobbs, P. Petrovic, J. L. Marchant, D. Hassabis, N. Weiskopf, B. Seymour, R. J. Dolan and C. D. Frith, *Science*, 2007, **317**, 1079–1083.
56. M. R. Liebowitz, A. J. Fyer, J. M. Gorman, D. Dillon, I. L. Appleby, G. Levy, S. Anderson, M. Levitt, M. Palij and S. O. Davies, *Arch. Gen. Psychiatry*, 1984, **41**, 764–770.
57. M. R. Liebowitz, J. M. Gorman, A. J. Fyer, M. Levitt, D. Dillon, G. Levy, I. L. Appleby, S. Anderson, M. Palij and S. O. Davies, *Arch. Gen. Psychiatry*, 1985, **42**, 709–719.
58. J. A. Den Boer, H. G. Westenberg, A. A. Klompmakers and L. E. van Lint, *Biol. Psychiatry*, 1989, **26**, 612–622.
59. E. Hollander, M. R. Liebowitz, J. M. Gorman, B. Cohen, A. Fyer and D. F. Klein, *Arch. Gen. Psychiatry*, 1989, **46**, 135–140.
60. S. W. Woods, D. S. Charney, J. Loke, W. K. Goodman, D. E. Redmond Jr and G. R. Heninger, *Arch. Gen. Psychiatry*, 1986, **43**, 900–909.
61. J. M. Gorman, M. R. Fyer, R. Goetz, J. Askanazi, M. R. Liebowitz, A. J. Fyer, J. Kinney and D. F. Klein, *Arch. Gen. Psychiatry*, 1988, **45**, 31–39.
62. L. A. Papp, D. F. Klein and J. M. Gorman, *Am. J. Psychiatry*, 1993, **150**, 1149–1157.
63. D. S. Charney, G. R. Heninger and A. Breier, *Arch. Gen. Psychiatry*, 1984, **41**, 751–763.
64. D. S. Charney and G. R. Heninger, *Arch. Gen. Psychiatry*, 1986, **43**, 1042–1054.
65. D. S. Charney and G. R. Heninger, *Arch. Gen. Psychiatry*, 1985, **42**, 458–467.
66. D. S. Charney and G. R. Heninger, *Arch. Gen. Psychiatry*, 1985, **42**, 473–481.
67. D. S. Charney, S. W. Woods, W. K. Goodman and G. R. Heninger, *Am. J. Psychiatry*, 1987, **144**, 1030–1036.
68. D. S. Charney, S. W. Woods, L. M. Nagy, S. M. Southwick, J. H. Krystal and G. R. Heninger, *J. Clin. Psychiatry*, 1990, **51**(A), 5–11.
69. R. S. Stewart, S. Devous MD, A. J. Rush, L. Lane and F. J. Bonte, *Am. J. Psychiatry*, 1988, **145**, 442–449.
70. E. M. Reiman, M. E. Raichle, E. Robins, M. A. Mintun, M. J. Fusselman, P. T. Fox, J. L. Price and K. A. Hackman, *Arch. Gen. Psychiatry*, 1989, **46**, 493–500.
71. S. W. Woods, K. Koster, J. K. Krystal, E. O. Smith, I. G. Zubal, P. B. Hoffer and D. S. Charney, *Lancet*, 1988, **2**, 678.



72. E. M. Reiman, M. E. Raichle, E. Robins, F. K. Butler, P. Herscovitch, P. Fox and J. Perlmutter, *Am. J. Psychiatry*, 1986, **143**, 469–477.
73. M. T. De Cristofaro, A. Sessarego, A. Pupi, F. Biondi and C. Faravelli, *Biol. Psychiatry*, 1993, **33**, 505–512.
74. T. E. Nordahl, W. E. Semple, M. Gross, T. A. Mellman, M. B. Stein, P. Goyer, A. C. King, T. W. Uhde and R. M. Cohen, *Neuropsychopharmacology*, 1990, **3**, 261–272.
75. A. Bisaga, J. L. Katz, A. Antonini, C. E. Wright, C. Margouleff, J. M. Gorman and D. Eidelberg, *Am. J. Psychiatry*, 1998, **155**, 1178–1183.
76. Y. Sakai, H. Kumano, M. Nishikawa, Y. Sakano, H. Kaiya, E. Imabayashi, T. Ohnishi, H. Matsuda, A. Yasuda, A. Sato, M. Diksic and T. Kuboki, *Neuroreport*, 2005, **16**, 927–931.
77. Y. Sakai, H. Kumano, M. Nishikawa, Y. Sakano, H. Kaiya, E. Imabayashi, T. Ohnishi, H. Matsuda, A. Yasuda, A. Sato, M. Diksic and T. Kuboki, *Neuroimage*, 2006, **33**, 218–226.
78. A. Bystritsky, D. Pontillo, M. Powers, F. W. Sabb, M. G. Craske and S. Y. Bookheimer, *Neuroreport*, 2001, **12**, 3953–3957.
79. R. J. Maddock, M. H. Buonocore, S. J. Kile and A. S. Garrett, *Neuroreport*, 2003, **14**, 325–328.
80. S. S. Pillay, S. A. Gruber, J. Rogowska, N. Simpson and D. A. Yurgelun-Todd, *J. Affect. Disord.*, 2006, **94**, 173–181.
81. S. S. Pillay, J. Rogowska, S. A. Gruber, N. Simpson and D. A. Yurgelun-Todd, *J. Anxiety Disord.*, 2007, **21**, 381–393.
82. N. Chechko, R. Wehrle, A. Erhardt, F. Holsboer, M. Czisch and P. G. Samann, *PLoS One*, 2009, **4**, e5537.
83. O. Tuescher, X. Protopopescu, H. Pan, M. Cloitre, T. Butler, M. Goldstein, J. C. Root, A. Engelien, D. Furman, M. Silverman, Y. Yang, J. Gorman, J. LeDoux, D. Silbersweig and E. Stern, *J. Anxiety Disord.*, 2011, **25**, 251–257.
84. K. Domschke, M. Braun, P. Ohrmann, T. Suslow, H. Kugel, J. Bauer, C. Hohoff, A. Kersting, A. Engelien, V. Arolt, W. Heindel and J. Deckert, *Int. J. Neuropsychopharmacol.*, 2006, **9**, 349–355.
85. K. Domschke, P. Ohrmann, M. Braun, T. Suslow, J. Bauer, C. Hohoff, A. Kersting, A. Engelien, V. Arolt, W. Heindel, J. Deckert and H. Kugel, *Psychiatry Res.*, 2008, **163**, 13–20.
86. M. Vythilingam, E. R. Anderson, A. Goddard, S. W. Woods, L. H. Staib, D. S. Charney and J. D. Bremner, *Psychiatry Res.*, 2000, **99**, 75–82.
87. R. R. Uchida, C. M. Del-Ben, A. C. Santos, D. Araujo, J. A. Crippa, F. S. Guimaraes and F. G. Graeff, *Braz. J. Med. Biol. Res.*, 2003, **36**, 925–929.
88. G. Massana, J. M. Serra-Grabulosa, P. Salgado-Pineda, C. Gasto, C. Junque, J. Massana and J. M. Mercader, *Am. J. Psychiatry*, 2003, **160**, 566–568.
89. G. Massana, J. M. Serra-Grabulosa, P. Salgado-Pineda, C. Gasto, C. Junque, J. Massana, J. M. Mercader, B. Gomez, A. Tobena and M. Salamero, *Neuroimage*, 2003, **19**, 80–90.

90. X. Protopopescu, H. Pan, O. Tuescher, M. Cloitre, M. Goldstein, A. Engelen, Y. Yang, J. Gorman, J. LeDoux, E. Stern and D. Silbersweig, *Neuroreport*, 2006, **17**, 361–363.
91. R. R. Uchida, C. M. Del-Ben, G. F. Busatto, F. L. Duran, F. S. Guimaraes, J. A. Crippa, D. Araujo, A. C. Santos and F. G. Graeff, *Psychiatry Res.*, 2008, **163**, 21–29.
92. A. Fujiwara, T. Yoshida, T. Otsuka, F. Hayano, T. Asami, H. Narita, M. Nakamura, T. Inoue and Y. Hirayasu, *Psychiatry Clin. Neurosci.*, 2011, **65**, 365–373.
93. S. Schlegel, H. Steinert, A. Bockisch, K. Hahn, R. Schloesser and O. Benkert, *Eur. Arch. Psychiatry Clin. Neurosci.*, 1994, **244**, 49–51.
94. W. Kaschka, H. Feistel and D. Ebert, *J. Psychiatr. Res.*, 1995, **29**, 427–434.
95. J. T. Kuikka, A. Pitkanen, U. Lepola, K. Partanen, P. Vainio, K. A. Bergstrom, H. J. Wieler, K. P. Kaiser, L. Mittelbach and H. Koponen, *Nucl. Med. Commun.*, 1995, **16**, 273–280.
96. J. D. Bremner, R. B. Innis, T. White, M. Fujita, D. Silbersweig, A. W. Goddard, L. Staib, E. Stern, A. Cappiello, S. Woods, R. Baldwin and D. S. Charney, *Biol. Psychiatry*, 2000, **47**, 96–106.
97. A. L. Malizia, V. J. Cunningham, C. J. Bell, P. F. Liddle, T. Jones and D. J. Nutt, *Arch. Gen. Psychiatry*, 1998, **55**, 715–720.
98. O. G. Cameron, G. C. Huang, T. Nichols, R. A. Koeppe, S. Minoshima, D. Rose and K. A. Frey, *Arch. Gen. Psychiatry*, 2007, **64**, 793–800.
99. G. Hasler, A. C. Nugent, P. J. Carlson, R. E. Carson, M. Geraci and W. C. Drevets, *Arch. Gen. Psychiatry*, 2008, **65**, 1166–1175.
100. A. Neumeister, E. Bain, A. C. Nugent, R. E. Carson, O. Bonne, D. A. Luckenbaugh, W. Eckelman, P. Herscovitch, D. S. Charney and W. C. Drevets, *J. Neurosci.*, 2004, **24**, 589–591.
101. A. W. Goddard, G. F. Mason, A. Almai, D. L. Rothman, K. L. Behar, O. A. Petroff, D. S. Charney and J. H. Krystal, *Arch. Gen. Psychiatry*, 2001, **58**, 556–561.
102. G. Hasler, J. W. van der Veen, M. Geraci, J. Shen, D. Pine and W. C. Drevets, *Biol. Psychiatry*, 2009, **65**, 273–275.
103. J. M. Hettema, C. A. Prescott, J. M. Myers, M. C. Neale and K. S. Kendler, *Arch. Gen. Psychiatry*, 2005, **62**, 182–189.
104. S. D. Norrholm and K. J. Ressler, *Neuroscience*, 2009, **164**, 272–287.
105. C. Rothe, D. Koszycki, J. Bradwejn, N. King, V. Deluca, S. Tharmalingam, F. Macciardi, J. Deckert and J. L. Kennedy, *Neuropsychopharmacology*, 2006, **31**, 2237–2242.
106. K. Domschke, J. Deckert, M. C. O'Donovan and S. J. Glatt, *Am. J. Med. Genet. B. Neuropsychiatr. Genet.*, 2007, **144B**, 667–673.
107. J. Samochowiec, A. Hajduk, A. Samochowiec, J. Horodnicki, G. Stepień, A. Grzywacz and J. Kucharska-Mazur, *Psychiatry Res.*, 2004, **128**, 21–26.
108. Y. Inada, H. Yoneda, J. Koh, J. Sakai, A. Himeji, Y. Kinoshita, K. Akabame, Y. Hiraoka and T. Sakai, *Psychiatry Res.*, 2003, **118**, 25–31.

109. E. Maron, T. Nikopensius, S. Koks, S. Altmae, E. Heinaste, K. Vabrit, V. Tammekivi, P. Hallast, K. Koido, A. Kurg, A. Metspalu, E. Vasar, V. Vasar and J. Shlik, *Psychiatr. Genet.*, 2005, **15**, 17–24.
110. E. Maron, I. Toru, A. Must, G. Tasa, E. Toover, V. Vasar, A. Lang and J. Shlik, *Neurosci. Lett.*, 2007, **411**, 180–184.
111. E. Maron, I. Toru, G. Tasa, A. Must, E. Toover, A. Lang, V. Vasar and J. Shlik, *Neurosci. Lett.*, 2008, **446**, 88–92.
112. Y. K. Kim, H. J. Lee, J. C. Yang, J. A. Hwang and H. K. Yoon, *Behav. Genet.*, 2009, **39**, 170–175.
113. S. Gyawali, R. Subaran, M. M. Weissman, D. Hershkowitz, M. C. McKenna, A. Talati, A. J. Fyer, P. Wickramaratne, P. B. Adams, S. E. Hodge, C. J. Schmidt, M. J. Bannon and C. E. Glatt, *Biol. Psychiatry*, 2010, **67**, 331–338.
114. L. J. Strug, R. Suresh, A. J. Fyer, A. Talati, P. B. Adams, W. Li, S. E. Hodge, T. C. Gilliam and M. M. Weissman, *Mol. Psychiatry*, 2010, **15**, 166–176.
115. S. P. Hamilton, S. L. Slager, A. B. De Leon, G. A. Heiman, D. F. Klein, S. E. Hodge, M. M. Weissman, A. J. Fyer and J. A. Knowles, *Neuropsychopharmacology*, 2004, **29**, 558–565.
116. S. P. Hamilton, S. L. Slager, G. A. Heiman, F. Haghighi, D. F. Klein, S. E. Hodge, M. M. Weissman, A. J. Fyer and J. A. Knowles, *Mol. Psychiatry*, 2000, **5**, 465–466.
117. S. P. Hamilton, S. L. Slager, L. Helleby, G. A. Heiman, D. F. Klein, S. E. Hodge, M. M. Weissman, A. J. Fyer and J. A. Knowles, *Mol. Psychiatry*, 2001, **6**, 59–65.
118. H. K. Yoon, J. C. Yang, H. J. Lee and Y. K. Kim, *J. Anxiety Disord.*, 2008, **22**, 1529–1534.
119. O. F. Olesen, B. Bennike, E. S. Hansen, P. Koefoed, D. P. Woldbye, T. G. Bolwig and E. Mellerup, *Psychiatr. Genet.*, 2005, **15**, 159.
120. A. J. Fyer, S. P. Hamilton, M. Durner, F. Haghighi, G. A. Heiman, R. Costa, O. Evgrafov, P. Adams, A. B. de Leon, N. Taveras, D. F. Klein, S. E. Hodge, M. M. Weissman and J. A. Knowles, *Biol. Psychiatry*, 2006, **60**, 388–401.
121. E. Maron, J. M. Hetttema and J. Shlik, *Mol. Psychiatry*, 2010, **15**, 681–701.
122. T. Otowa, E. Yoshida, N. Sugaya, S. Yasuda, Y. Nishimura, K. Inoue, M. Tochigi, T. Umekage, T. Miyagawa, N. Nishida, K. Tokunaga, H. Tani, T. Sasaki, H. Kaiya and Y. Okazaki, *J. Hum. Genet.*, 2009, **54**, 122–126.
123. T. Otowa, H. Tani, N. Sugaya, E. Yoshida, K. Inoue, S. Yasuda, T. Shimada, Y. Kawamura, M. Tochigi, T. Minato, T. Umekage, T. Miyagawa, N. Nishida, K. Tokunaga, Y. Okazaki, H. Kaiya and T. Sasaki, *J. Hum. Genet.*, 2010, **55**, 91–96.
124. A. Erhardt, L. Cizibere, D. Roeske, S. Lucae, P. G. Unschuld, S. Ripke, M. Specht, M. A. Kohli, S. Kloiber, M. Ising, A. Heck, H. Pfister, P. Zimmermann, R. Lieb, B. Putz, M. Uhr, P. Weber, J. M. Deussing,

- M. Gonik, M. Bunck, M. S. Kessler, E. Frank, C. Hohoff, K. Domschke, P. Krakowitzky, W. Maier, B. Bandelow, C. Jacob, J. Deckert, S. Schreiber, J. Strohmaier, M. Nothen, S. Cichon, M. Rietschel, T. Bettecken, M. E. Keck, R. Landgraf, B. Muller-Myhsok, F. Holsboer and E. B. Binder, *Mol. Psychiatry*, 2011, **16**, 647–663.
125. C. Blaya, G. A. Salum, P. Moorjani, A. C. Seganfredo, E. Heldt, S. Leistner-Segal, J. W. Smoller and G. G. Manfro, *Neurosci. Lett.*, 2010, **485**, 11–15.
126. N. Gregersen, H. A. Dahl, H. N. Buttenschon, M. Nyegaard, A. Hedemand, T. D. Als, A. G. Wang, S. Joensen, D. P. Woldbye, P. Koefoed, A. S. Kristensen, T. A. Kruse, A. D. Borglum and O. Mors, *Eur. J. Hum. Genet.*, 2011, **20**(1), 84.
127. K. Annerbrink, L. Westberg, M. Olsson, S. Andersch, I. Sjodin, G. Holm, C. Allgulander and E. Eriksson, *Psychiatr. Genet.*, 2011, **21**, 85–89.
128. M. Muinos-Gimeno, Y. Espinosa-Parrilla, M. Guidi, B. Kagerbauer, T. Sipila, E. Maron, K. Pettai, L. Kananen, R. Navines, R. Martin-Santos, M. Gratacos, A. Metspalu, I. Hovatta and X. Estivill, *Biol. Psychiatry*, 2011, **69**, 526–533.
129. L. M. Shin and I. Liberzon, *Neuropsychopharmacology*, 2010, **35**, 169–191.
130. S. Yamamoto, S. Morinobu, S. Takei, M. Fuchikami, A. Matsuki, S. Yamawaki and I. Liberzon, *Depress. Anxiety*, 2009, **26**, 1110–1117.
131. I. Liberzon, M. Krstov and E. A. Young, *Psychoneuroendocrinology*, 1997, **22**, 443–453.
132. I. Liberzon, J. F. Lopez, S. B. Flagel, D. M. Vazquez and E. A. Young, *J. Neuroendocrinol.*, 1999, **11**, 11–17.
133. T. Takahashi, S. Morinobu, Y. Iwamoto and S. Yamawaki, *Psychopharmacology (Berl.)*, 2006, **189**, 165–173.
134. S. Yamamoto, S. Morinobu, M. Fuchikami, A. Kurata, T. Kozuru and S. Yamawaki, *Neuropsychopharmacology*, 2008, **33**, 2108–2116.
135. R. Yehuda, S. M. Southwick, J. H. Krystal, D. Bremner, D. S. Charney and J. W. Mason, *Am. J. Psychiatry*, 1993, **150**, 83–86.
136. R. Yehuda, D. Boissoneau, M. T. Lowy and E. L. Giller Jr, *Arch. Gen. Psychiatry*, 1995, **52**, 583–593.
137. R. Yehuda, J. A. Golier, S. L. Halligan, M. Meaney and L. M. Bierer, *Am. J. Psychiatry*, 2004, **161**, 1397–1403.
138. B. H. Harvey, F. Oosthuizen, L. Brand, G. Wegener and D. J. Stein, *Psychopharmacology (Berl.)*, 2004, **175**, 494–502.
139. E. Geuze, B. N. van Berckel, A. A. Lammertsma, R. Boellaard, C. S. de Kloet, E. Vermetten and H. G. Westenberg, *Mol. Psychiatry*, 2008, **13**, 74–83, 3.
140. E. R. Klaassens, E. J. Giltay, P. Cuijpers, T. van Veen and F. G. Zitman, *Psychoneuroendocrinology*, 2012, **37**(3), 317.
141. R. Yehuda, W. Blair, E. Labinsky and L. M. Bierer, *Am. J. Psychiatry*, 2007, **164**, 163–166.
142. R. Yehuda, M. H. Teicher, J. R. Seckl, R. A. Grossman, A. Morris and L. M. Bierer, *Arch. Gen. Psychiatry*, 2007, **64**, 1040–1048.

143. S. M. Southwick, J. H. Krystal, C. A. Morgan, D. Johnson, L. M. Nagy, A. Nicolaou, G. R. Heninger and D. S. Charney, *Arch. Gen. Psychiatry*, 1993, **50**, 266–274.
144. S. M. Southwick, J. H. Krystal, J. D. Bremner, C. A. Morgan 3rd, A. L. Nicolaou, L. M. Nagy, D. R. Johnson, G. R. Heninger and D. S. Charney, *Arch. Gen. Psychiatry*, 1997, **54**, 749–758.
145. T. D. Geraciotti Jr, D. G. Baker, N. N. Ekhtator, S. A. West, K. K. Hill, A. B. Bruce, D. Schmidt, B. Rounds-Kugler, R. Yehuda, P. E. Keck Jr and J. W. Kasckow, *Am. J. Psychiatry*, 2001, **158**, 1227–1230.
146. A. M. Rasmusson, R. L. Hauger, C. A. Morgan, J. D. Bremner, D. S. Charney and S. M. Southwick, *Biol. Psychiatry*, 2000, **47**, 526–539.
147. R. Yehuda, S. Brand and R. K. Yang, *Biol. Psychiatry*, 2006, **59**, 660–663.
148. R. Sah, N. N. Ekhtator, J. R. Strawn, F. R. Sallee, D. G. Baker, P. S. Horn and T. D. Geraciotti Jr, *Biol. Psychiatry*, 2009, **66**, 705–707.
149. T. D. Geraciotti Jr, L. L. Carpenter, M. J. Owens, D. G. Baker, N. N. Ekhtator, P. S. Horn, J. R. Strawn, G. Sanacora, B. Kinkead, L. H. Price and C. B. Nemeroff, *Am. J. Psychiatry*, 2006, **163**, 637–643.
150. S. J. Mathew, M. Vythilingam, J. W. Murrough, C. A. Zarate Jr, A. Feder, D. A. Luckenbaugh, B. Kinkead, M. K. Parides, D. G. Trist, M. S. Bani, P. U. Bettica, E. M. Ratti and D. S. Charney, *Eur. Neuropsychopharmacol.*, 2011, **21**, 221–229.
151. J. R. Strawn, G. J. Pyne-Geithman, N. N. Ekhtator, P. S. Horn, T. W. Uhde, L. A. Shutter, D. G. Baker and T. D. Geraciotti Jr, *Psychoneuroendocrinology*, 2010, **35**, 1001–1007.
152. S. L. Rauch, P. J. Whalen, L. M. Shin, S. C. McInerney, M. L. Macklin, N. B. Lasko, S. P. Orr and R. K. Pitman, *Biol. Psychiatry*, 2000, **47**, 769–776.
153. L. M. Shin, S. P. Orr, M. A. Carson, S. L. Rauch, M. L. Macklin, N. B. Lasko, P. M. Peters, L. J. Metzger, D. D. Dougherty, P. A. Cannistraro, N. M. Alpert, A. J. Fischman and R. K. Pitman, *Arch. Gen. Psychiatry*, 2004, **61**, 168–176.
154. L. M. Shin, C. I. Wright, P. A. Cannistraro, M. M. Wedig, K. McMullin, B. Martis, M. L. Macklin, N. B. Lasko, S. R. Cavanagh, T. S. Krangel, S. P. Orr, R. K. Pitman, P. J. Whalen and S. L. Rauch, *Arch. Gen. Psychiatry*, 2005, **62**, 273–281.
155. J. D. Bremner, E. Vermetten, C. Schmahl, V. Vaccarino, M. Vythilingam, N. Afzal, C. Grillon and D. S. Charney, *Psychol. Med.*, 2005, **35**, 791–806.
156. A. Etkin and T. D. Wager, *Am. J. Psychiatry*, 2007, **164**, 1476–1488.
157. I. Liberzon and C. S. Sripada, *Prog. Brain Res.*, 2008, **167**, 151–169.
158. S. L. Rauch, B. A. van der Kolk, R. E. Fisler, N. M. Alpert, S. P. Orr, C. R. Savage, A. J. Fischman, M. A. Jenike and R. K. Pitman, *Arch. Gen. Psychiatry*, 1996, **53**, 380–387.
159. L. M. Shin, S. M. Kosslyn, R. J. McNally, N. M. Alpert, W. L. Thompson, S. L. Rauch, M. L. Macklin and R. K. Pitman, *Arch. Gen. Psychiatry*, 1997, **54**, 233–241.

160. L. M. Shin, R. J. McNally, S. M. Kosslyn, W. L. Thompson, S. L. Rauch, N. M. Alpert, L. J. Metzger, N. B. Lasko, S. P. Orr and R. K. Pitman, *Am. J. Psychiatry*, 1999, **156**, 575–584.
161. J. D. Bremner, L. H. Staib, D. Kaloupek, S. M. Southwick, R. Soufer and D. S. Charney, *Biol. Psychiatry*, 1999, **45**, 806–816.
162. L. M. Shin, C. I. Wright, P. A. Cannistraro, M. M. Wedig, K. McMullin, B. Martis, M. L. Macklin, N. B. Lasko, S. R. Cavanagh, T. S. Krangel, S. P. Orr, R. K. Pitman, P. J. Whalen and S. L. Rauch, *Arch. Gen. Psychiatry*, 2005, **62**, 273–281.
163. J. D. Bremner, M. Narayan, L. H. Staib, S. M. Southwick, T. McGlashan and D. S. Charney, *Am. J. Psychiatry*, 1999, **156**, 1787–1795.
164. R. A. Lanius, P. C. Williamson, M. Densmore, K. Boksman, M. A. Gupta, R. W. Neufeld, J. S. Gati and R. S. Menon, *Am. J. Psychiatry*, 2001, **158**, 1920–1922.
165. R. A. Bryant, K. Felmingham, A. Kemp, P. Das, G. Hughes, A. Peduto and L. Williams, *Psychol. Med.*, 2008, **38**, 555–561.
166. L. M. Shin, S. P. Orr, M. A. Carson, S. L. Rauch, M. L. Macklin, N. B. Lasko, P. M. Peters, L. J. Metzger, D. D. Dougherty, P. A. Cannistraro, N. M. Alpert, A. J. Fischman and R. K. Pitman, *Arch. Gen. Psychiatry*, 2004, **61**, 168–176.
167. R. A. Lanius, P. C. Williamson, J. Hopper, M. Densmore, K. Boksman, M. A. Gupta, R. W. Neufeld, J. S. Gati and R. S. Menon, *Biol. Psychiatry*, 2003, **53**, 204–210.
168. K. L. Phan, J. C. Britton, S. F. Taylor, L. M. Fig and I. Liberzon, *Arch. Gen. Psychiatry*, 2006, **63**, 184–192.
169. R. A. Bryant, K. L. Felmingham, A. H. Kemp, M. Barton, A. S. Peduto, C. Rennie, E. Gordon and L. M. Williams, *Biol. Psychiatry*, 2005, **58**, 111–118.
170. I. Liberzon, A. P. King, J. C. Britton, K. L. Phan, J. L. Abelson and S. F. Taylor, *Am. J. Psychiatry*, 2007, **164**, 1250–1258.
171. J. D. Bremner, P. Randall, T. M. Scott, R. A. Bronen, J. P. Seibyl, S. M. Southwick, R. C. Delaney, G. McCarthy, D. S. Charney and R. B. Innis, *Am. J. Psychiatry*, 1995, **152**, 973–981.
172. J. D. Bremner, P. Randall, E. Vermetten, L. Staib, R. A. Bronen, C. Mazure, S. Capelli, G. McCarthy, R. B. Innis and D. S. Charney, *Biol. Psychiatry*, 1997, **41**, 23–32.
173. J. D. Bremner, M. Vythilingam, E. Vermetten, S. M. Southwick, T. McGlashan, A. Nazeer, S. Khan, L. V. Vaccarino, R. Soufer, P. K. Garg, C. K. Ng, L. H. Staib, J. S. Duncan and D. S. Charney, *Am. J. Psychiatry*, 2003, **160**, 924–932.
174. M. E. Molina, R. Isoardi, M. N. Prado and S. Bentolila, *World J. Biol. Psychiatry*, 2007, **11**, 1–9.
175. O. Bonne, D. Brandes, A. Gilboa, J. M. Gomeri, M. E. Shenton, R. K. Pitman and A. Y. Shalev, *Am. J. Psychiatry*, 2001, **158**, 1248–1251.
176. L. A. Tupler and M. D. De Bellis, *Biol. Psychiatry*, 2006, **59**, 523–529.



177. E. Geuze, H. G. Westenberg, A. Jochims, C. S. de Kloet, M. Bohus, E. Vermetten and C. Schmahl, *Arch. Gen. Psychiatry*, 2007, **64**, 76–85.
178. E. Geuze, E. Vermetten, M. Ruf, C. S. de Kloet and H. G. Westenberg, *J. Psychiatr. Res.*, 2008, **42**, 659–669.
179. S. H. Woodward, D. G. Kaloupek, C. C. Streeter, C. Martinez, M. Schaer and S. Eliez, *Biol. Psychiatry*, 2006, **59**, 582–587.
180. R. J. Schulz-Heik, M. Schaer, S. Eliez, J. F. Hallmayer, X. Lin, D. G. Kaloupek and S. H. Woodward, *Biol. Psychiatry*, 2011, **70**(11), 1091.
181. J. D. Bremner, R. B. Innis, S. M. Southwick, L. Staib, S. Zoghbi and D. S. Charney, *Am. J. Psychiatry*, 2000, **157**, 1120–1126.
182. M. Fujita, S. M. Southwick, C. C. Denucci, S. S. Zoghbi, M. S. Dillon, R. M. Baldwin, A. Bozkurt, A. Kugaya, N. P. Verhoeff, J. P. Seibyl and R. B. Innis, *Biol. Psychiatry*, 2004, **56**, 95–100.
183. O. Bonne, E. Bain, A. Neumeister, A. C. Nugent, M. Vythilingam, R. E. Carson, D. A. Luckenbaugh, W. Eckelman, P. Herscovitch, W. C. Drevets and D. S. Charney, *Am. J. Psychiatry*, 2005, **162**, 383–385.
184. I. Liberzon, S. F. Taylor, K. L. Phan, J. C. Britton, L. M. Fig, J. A. Bueller, R. A. Koeppe and J. K. Zubieta, *Biol. Psychiatry*, 2007, **61**, 1030–1038.
185. J. W. Murrough, C. Czermak, S. Henry, N. Nabulsi, J. D. Gallezot, R. Gueorguieva, B. Planeta-Wilson, J. H. Krystal, J. F. Neumaier, Y. Huang, Y. S. Ding, R. E. Carson and A. Neumeister, *Arch. Gen. Psychiatry*, 2011, **68**, 892–900.
186. J. W. Murrough, Y. Huang, J. Hu, S. Henry, W. Williams, J. D. Gallezot, C. R. Bailey, J. H. Krystal, R. E. Carson and A. Neumeister, *Biol. Psychiatry*, 2011, **70**(11), 1033.
187. M. B. Stein, K. L. Jang, S. Taylor, P. A. Vernon and W. J. Livesley, *Am. J. Psychiatry*, 2002, **159**, 1675–1681.
188. W. R. True, J. Rice, S. A. Eisen, A. C. Heath, J. Goldberg, M. J. Lyons and J. Nowak, *Arch. Gen. Psychiatry*, 1993, **50**, 257–264.
189. J. W. Murrough and D. S. Charney, *Biol. Psychiatry*, 2011, **69**, 510–512.
190. H. J. Lee, M. S. Lee, R. H. Kang, H. Kim, S. D. Kim, B. S. Kee, Y. H. Kim, Y. K. Kim, J. B. Kim, B. K. Yeon, K. S. Oh, B. H. Oh, J. S. Yoon, C. Lee, H. Y. Jung, I. S. Chee and I. H. Paik, *Depress. Anxiety*, 2005, **21**, 135–139.
191. D. G. Kilpatrick, K. C. Koenen, K. J. Ruggiero, R. Acierno, S. Galea, H. S. Resnick, J. Roitzsch, J. Boyle and J. Gelernter, *Am. J. Psychiatry*, 2007, **164**, 1693–1699.
192. P. Xie, H. R. Kranzler, J. Poling, M. B. Stein, R. F. Anton, K. Brady, R. D. Weiss, L. Farrer and J. Gelernter, *Arch. Gen. Psychiatry*, 2009, **66**, 1201–1209.
193. I. T. Kolassa, V. Ertl, C. Eckart, F. Glockner, S. Kolassa, A. Papassotiropoulos, D. J. de Quervain and T. Elbert, *J. Clin. Psychiatry*, 2010, **71**, 543–547.
194. R. A. Bryant, K. L. Felmingham, E. M. Falconer, L. Pe Benito, C. Dobson-Stone, K. D. Pierce and P. R. Schofield, *Biol. Psychiatry*, 2010, **67**, 1217–1219.



195. E. B. Binder, R. G. Bradley, W. Liu, M. P. Epstein, T. C. Deveau, K. B. Mercer, Y. Tang, C. F. Gillespie, C. M. Heim, C. B. Nemeroff, A. C. Schwartz, J. F. Cubells and K. J. Ressler, *JAM*, 2008, **299**, 1291–1305.
196. K. J. Ressler, K. B. Mercer, B. Bradley, T. Jovanovic, A. Mahan, K. Kerley, S. D. Norrholm, V. Kilaru, A. K. Smith, A. J. Myers, M. Ramirez, A. Engel, S. E. Hammack, D. Toufexis, K. M. Braas, E. B. Binder and V. May, *Nature*, 2011, **470**, 492–497.
197. T. Jovanovic and K. J. Ressler, *Am. J. Psychiatry*, 2010, **167**, 648–662.
198. L. L. McGhee, C. V. Maani, T. H. Garza, K. M. Gaylord and I. H. Black, *J. Trauma*, 2008, **64**, S195–S198; Discussion S197–S198.
199. J. H. Krystal, S. J. Mathew, D. C. D'souza, A. Garakani, H. Gunduz-Bruce and D. S. Charney, *CNS Drugs*, 2010, **24**, 669–693.
200. R. Rupprecht, G. Rammes, D. Eser, T. C. Baghai, C. Schule, C. Nothdurfter, T. Troxler, C. Gentsch, H. O. Kalkman, F. Chaperon, V. Uzunov, K. H. McAllister, V. Bertaina-Anglade, C. D. La Rochelle, D. Tuerck, A. Floesser, B. Kiese, M. Schumacher, R. Landgraf, F. Holsboer and K. Kucher, *Science*, 2009, **325**, 490–493.
201. C. A. Morgan 3rd, S. Wang, S. M. Southwick, A. Rasmusson, G. Hazlett, R. L. Hauger and D. S. Charney, *Biol. Psychiatry*, 2000, **47**, 902–909.
202. C. A. Morgan 3rd, A. M. Rasmusson, S. Wang, G. Hoyt, R. L. Hauger and G. Hazlett, *Biol. Psychiatry*, 2002, **52**, 136–142.
203. A. R. Gutman, Y. Yang, K. J. Ressler and M. Davis, *J. Neurosci.*, 2008, **28**, 12682–12690.
204. T. J. Sajdyk, P. L. Johnson, R. J. Leitemann, S. D. Fitz, A. Dietrich, M. Morin, D. R. Gehlert, J. H. Urban and A. Shekhar, *J. Neurosci.*, 2008, **28**, 893–903.
205. Z. Zhou, G. Zhu, A. R. Hariri, M. A. Enoch, D. Scott, R. Sinha, M. Virkkunen, D. C. Mash, R. H. Lipsky, X. Z. Hu, C. A. Hodgkinson, K. Xu, B. Buzas, Q. Yuan, P. H. Shen, R. E. Ferrell, S. B. Manuck, S. M. Brown, R. L. Hauger, C. S. Stohler, J. K. Zubieta and D. Goldman, *Nature*, 2008, **452**, 997–1001.
206. B. J. Mickey, Z. Zhou, M. M. Heitzeg, E. Heinz, C. A. Hodgkinson, D. T. Hsu, S. A. Langenecker, T. M. Love, M. Pecina, T. Shafir, C. S. Stohler, D. Goldman and J. K. Zubieta, *Arch. Gen. Psychiatry*, 2011, **68**, 158–166.
207. T. Sakurai, *Nat. Rev. Neurosci.*, 2007, **8**, 171–181.

## CHAPTER 10

# *Glutamate Approaches Towards the Treatment of Mood Disorders*

CARRIE K. JONES, P. JEFFREY CONN AND  
CRAIG W. LINDSLEY\*

Vanderbilt University Medical School, Department of Pharmacology,  
Vanderbilt Center for Neuroscience Drug Discovery, 1205 LH, Nashville,  
TN 37232-0697, USA

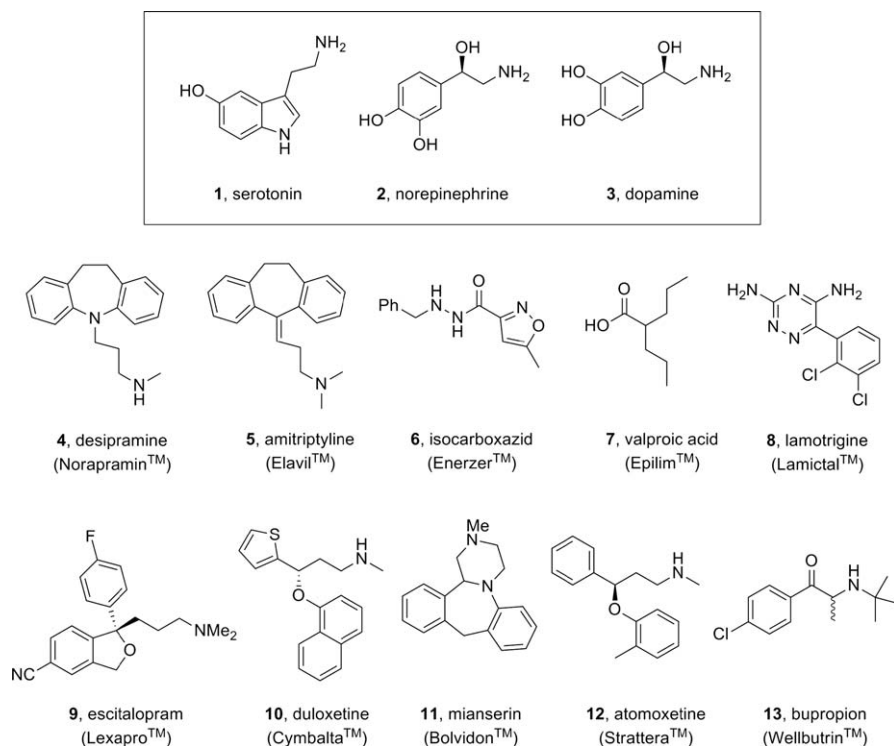
\*Email: [craig.lindsley@vanderbilt.edu](mailto:craig.lindsley@vanderbilt.edu)

## 10.1 Introduction to Mood Disorders

Mood disorders are prevalent, chronic forms of psychiatric illness that affect millions of people worldwide, with the risk of acquiring a mood disorder exceeding 20% in the USA alone.<sup>1</sup> With onset in childhood or adolescence, mood disorders are associated with recurring episodes of symptoms, lingering symptoms between episodes and severe functional impairments with a high risk of suicide.<sup>2–4</sup> First coined “affective disorder” by the English psychiatrist Maudsley, the DSM-IV now utilizes the clinical term “mood disorder” to designate a group of diagnoses where a disturbance in a person’s mood is the central feature.<sup>5</sup> Mood disorders encompass bipolar disorder or BPD (formerly manic depression) and major depressive disorder or MDD (formerly referred to as both major depression and clinical depression). BPD is characterized by alternating cycles of deep depression and mania, often accompanied by

hallucinations and delusions. MDD is forecasted by the WHO Global Burden of Disease to be the second leading cause of disability worldwide by 2020, and is associated with two characteristic symptoms: depressed mood and anhedonia.<sup>6</sup> Alterations in normal affective response results in the disruptions of many daily life functions, including occupational and/or social impairments, weight changes, sleep disturbances, fatigue, feelings of worthlessness or guilt, recurrent thoughts of death or suicide and psychomotor agitation or retardation.<sup>7</sup>

In the 1950s and 1960s, MDD and BPD patients were treated with opioids, amphetamines and the monovalent cation lithium (still used today as a front-line therapy for BPD). Introduction of monoamine oxidase inhibitors and tricyclic antidepressants in the late 1950s and 1960s supplanted use of opioids and amphetamines for treatment of major depression and predecessors of the range of antidepressant medications available today.<sup>8</sup> The prevailing hypothesis for the pathophysiology of MDD for the past 20 years has been the “biogenic amine depletion hypothesis”, which states that the symptoms of depression are due to decreases in the synaptic availability of biogenic amines serotonin (5-HT, **1**), norepinephrine (NE, **2**) and dopamine (DA, **3**) in subcortical brain regions (Figure 10.1).<sup>8</sup> This hypothesis is based on the fact that all clinically available antidepressants, including tricyclic antidepressants (TCAs, **4** and **5**), monoamine oxidase inhibitors (MAOIs, **6**), mood stabilizers (**7** and **8**), selective serotonin reuptake inhibitors (SSRIs, **9**), serotonin-norepinephrine reuptake inhibitors (SNRIs, **10**), noradrenergic and specific serotonergic antidepressants (NaSSAs, **11**), norepinephrine reuptake inhibitors (NRIs, **12**) and norepinephrine-dopamine reuptake inhibitors (NDRIs, **13**), produce significant enhancement of synaptic biogenic amine levels (Figure 10.1).<sup>8</sup> However, current antidepressant therapies require a minimum of 3 to 4 weeks for onset of action and produce symptomatic remission in only one-third of depressed patients with their first course of medication, according to the STAR\*D open-label study.<sup>9–11</sup> For patients that do respond to initial treatment regimens, fewer than half reach sustained remission or become symptom-free.<sup>10–11</sup> The delayed onset of antidepressant effects can also negatively impact compliance and have severe consequences for MDD patients suffering from suicidal ideations resulting in a 15% suicide rate.<sup>12</sup> More recently, the delayed onset of clinical efficacy has led to speculations that downstream neural adaptations, such as changes in the brain-derived neurotrophic factor (BDNF)-TrkB receptor signalling pathway, rather than the acute elevation in synaptic biogenic amine levels may be responsible for therapeutic effects of clinically available antidepressants.<sup>13</sup> Taken together, there remains a critical unmet need to develop novel therapeutic strategies for the treatment of MDD with more rapid onsets of action, higher rates of response and remission for both depressed mood and anhedonic symptoms and enhanced side-effect profiles. More recently, both preclinical and clinical studies have provided strong data sets supporting the involvement of the glutamatergic system in the treatment of mood disorders, as attention has shifted from a



**Figure 10.1** Structures of the monoamine neurotransmitters serotonin (**1**, 5-HT), norepinephrine (**2**, NA), dopamine (**3**, DA). Structures of classical mood disorder therapeutics: tricyclic antidepressants **4** and **5**, monoamine oxidase inhibitor (MAOI) **6**, anticonvulsants **7** and **8**, selective serotonin reuptake inhibitor (SSRI) **9**, serotonin-norepinephrine reuptake inhibitor (SNRI) **10**, noradrenergic and specific serotonergic antidepressant **11**, norepinephrine reuptake inhibitor (NRI) **12** and norepinephrine-dopamine reuptake inhibitor (NDRI) **13**.

focus on absolute changes in monoamines to the role of neural circuits and systems that control synaptic and neural plasticity.

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) and is responsible for generation of fast excitatory synaptic responses at the vast majority of CNS synapses.<sup>14</sup> Substantial evidence now suggests that abnormal function of the glutamatergic system is a key contributor to impairments in synaptic and neural plasticity observed in MDD patients, as well as a number of other CNS pathologies.<sup>8</sup> Multiple studies have reported that glutamate levels are significantly elevated in the plasma, cerebrospinal fluid and frontal cortex of patients with MDD and that there is a positive correlation between plasma glutamate levels and severity of depressive symptoms.<sup>15–16</sup> These findings have also been

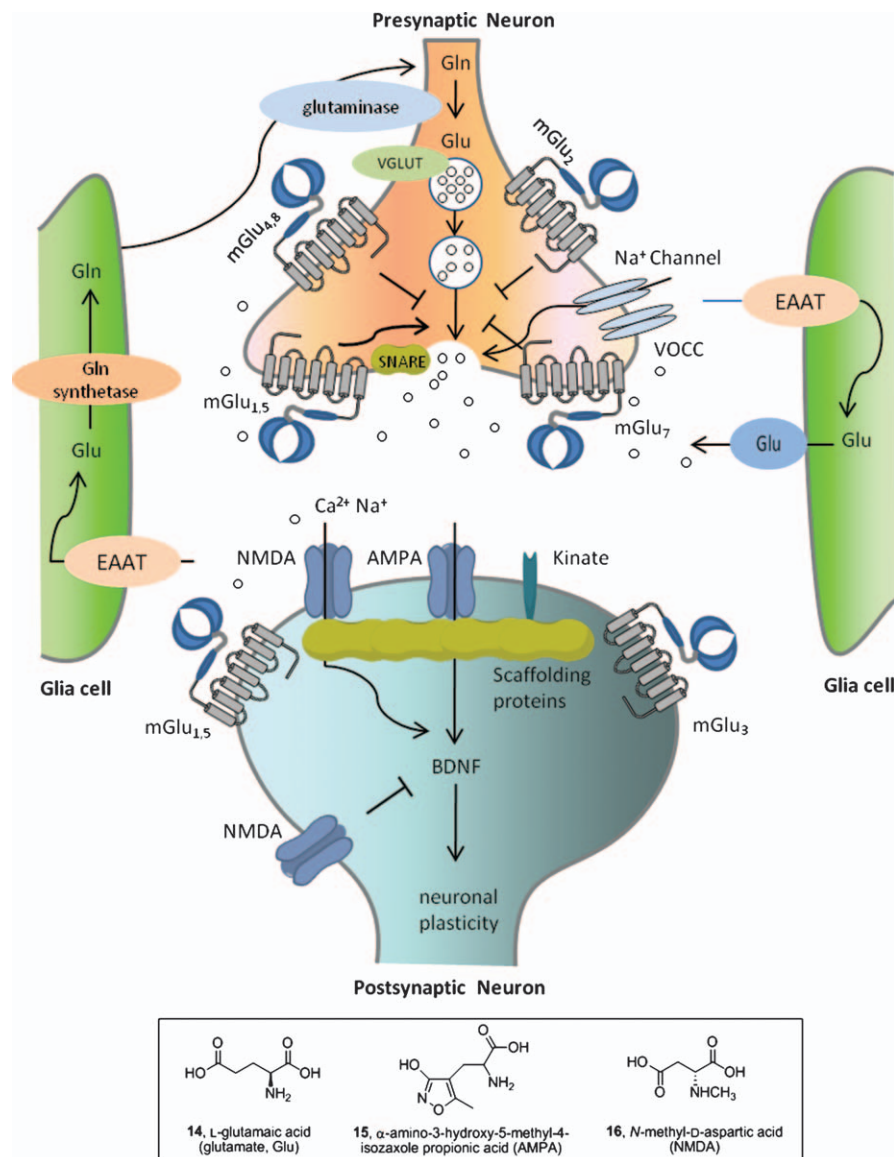
confirmed in *post mortem* studies with MDD patients. Chronic antidepressant treatment (4–5 weeks) produces significant decreases in plasma levels of glutamate and up-regulation of forebrain glutamate receptor subunits (e.g. NR2A, GluR1, GluR2) suggesting that delayed structural and molecular adaptations in glutamatergic synapses may be linked to the action of chronic antidepressant exposure.<sup>17–18</sup> Moreover, acute behavioral stress, which is a major risk factor for MDD, is associated with increased glutamatergic neurotransmission in forebrain regions and can also be reduced with chronic antidepressant treatment.<sup>19–21</sup> In addition to changes in glutamate levels and glutamate receptor alterations, there is a significant body of work indicating glial-cell pathology in MDD and effects of mood disorder therapeutics impacting the glutamatergic system. Collectively, these studies suggest that pharmacologic approaches that reduce glutamatergic signalling may represent an important alternative approach for the treatment of symptoms associated with mood disorders.

## 10.2 Glutamatergic Neurotransmission

Due to the critical role of glutamate in the CNS for neuronal function and the need to protect glial cells and surrounding neurons from the toxic effects of glutamate over-exposure, tight control of glutamatergic neurotransmission is necessary. Referred to as the “tripartite glutamatergic synapse” (Figure 10.2), consisting of pre-synaptic and post-synaptic neurons and glial cells, these neurons control synaptic and extra-synaptic glutamate levels through an integrated network of receptors, proteins ion channels and transporters.<sup>22</sup> Many of these (*vide infra*) represent druggable targets for the treatment of mood disorders. Within the tripartite glutamatergic synapse, glutamate is recycled through the glutamate/glutamine cycle.<sup>23</sup> Once generated, glutamate is transported into the synaptic vesicles by vesicular glutamate transporters (VGLUTs), where it is stored at high concentrations.<sup>24–25</sup> Glutamate is then released in an activity-dependent manner into the synapse by complex interactions with *N*-ethylmaleimide-sensitive factor receptor (SNARE) proteins. Upon release, synaptic glutamate binds to and activates ionotropic (AMPA, NMDA and kainate) and metabotropic glutamate (mGlu) receptors.<sup>13,26</sup> Glutamate is then cleared from the synapse and into glial cells by the action of high-affinity excitatory amino acid transporters (EAATs).<sup>22</sup> Glutamate uptake by EAATs is the primary mechanism for glutamate clearance from the synapse and terminating the action of glutamate. When EAATs fail to clear glutamate, cellular damage and toxicity result, and excessive glutamate is linked to mood disorders.<sup>27–28</sup> Once in the glial cells, glutamate is converted back into glutamine by the action of glutamine synthetase, and glutamine is then shuttled back into the pre-synaptic glutamatergic neuron, where glutaminase hydrolyses it back into glutamate.<sup>22</sup>

## 10.3 Glutamatergic Targets for the Treatment of Mood Disorders

Based on the observed changes in glutamate levels and glutamate receptor expression (*i.e.* NMDA and AMPA) and decreased number of glial cells and/or reduced expression of EAATs in *post mortem* studies, dysfunction in the glutamatergic system is now central to the etiology of mood disorders.<sup>8,22</sup> Recently, studies with the classical mood disorder therapeutics have shown



downstream effects on the glutamatergic system. For example, Skolnick and coworkers demonstrated that classical tricyclic antidepressants, reuptake inhibitors and even electroconvulsive therapy regulate NMDA receptor expression and function and in some cases increased expression of the GLUR1 subunit of the AMPA receptor.<sup>29–34</sup> Antidepressants, such as fluoxetine, have also been shown to increase AMPA receptor function by facilitating AMPA receptor subunit phosphorylation.<sup>35</sup> Mood stabilizers also have effects on the glutamatergic system, with both lithium and valproic acid increasing synaptosomal glutamate uptake by increasing the levels of EAATs and impacting AMPA trafficking.<sup>36–39</sup> Combined, all of these data suggest a number of discrete molecular targets in the glutamatergic system that could be targeted for development of novel mood disorder therapeutics.

### 10.3.1 Inhibitor of Glutamate Release

The anticonvulsant (and BPD therapeutic) lamotrigine **8** has demonstrated the most direct effects on the glutamatergic system, by inhibiting the release of glutamate in rat hippocampus and increasing AMPA receptor expression.<sup>40</sup> Heralded as one of two prototypes (ketamine is the other, *vide infra*) for the next generation of antidepressants and mood stabilizers, riluzole **17** (Figure 10.3) is a modulator of glutamate release with proven neuroprotective properties approved by the FDA for amyotrophic lateral sclerosis (ALS). By enhancing membrane insertion of GluR1 and GluR2 of AMPA receptors, riluzole increases AMPA trafficking and inhibits glutamate release.<sup>41</sup> However, the antidepressant effects of riluzole, demonstrated in both preclinical and clinical studies, are believed to be the result of enhancing glutamate uptake in glial cells *via* EAATs, and thereby preventing cellular excitotoxicity.<sup>42–45</sup> The first open-label trial involved patients that failed to respond to at least two antidepressants. Here, riluzole showed significant antidepressant efficacy, with 46% of enrolled patients

---

**Figure 10.2** Glutamatergic neurotransmission and potential glutamate-based therapeutic targets for mood disorders (MDD and BPD), with the structures of L-glutamic acid **14** (L-glutamate or Glu) the major excitatory neurotransmitter in the mammalian CNS, AMPA **15** and NMDA **16**, highlighted in the inset. Within the pre-synaptic neuron, Glu is generated by the action of glutaminase on glutamine, and then packaged into vesicles by VGLUTs. **Pre-synaptic targets for mood disorders:** 1) modulation of VGLUTs, 2) activation of either voltage-gated sodium channels or VOCCs leads to fusion of vesicles and release of Glu into the synaptic cleft, 3) activation of pre-synaptic mGlu2 directly reduces the release of Glu through a feedback inhibition mechanism and possibly mGlu7. **Glia targets for mood disorders:** 1) directly block release of Glu from glia and 2) potentiation of the activity of the EAATs (EAAT1 or GLAST and EAAT2 or GLT-1). **Post-synaptic targets for mood disorders:** 1) direct and/or indirect inhibitors of the NMDA receptor, 2) activators of AMPA receptors, 3) kainate (KA) receptors that control GABA release, 4) the metabotropic glutamate (mGlu) receptors and 5) scaffolding proteins that physically and functionally link various post-synaptic receptors.





glutamate uptake, *via* EAATs, might have antidepressant effects suggests that small molecules capable of enhancing EAAT activity may have therapeutic potential. Early data supporting this comes from  $\beta$ -lactam antibiotics used to treat neurodegenerative diseases and the mood stabilizers lithium and valproic acid **7**.<sup>46–48</sup> The antibiotic ceftriaxone **18** has been shown to enhance the expression of the gene encoding to EAAT2 and, as a result, displays efficacy in multiple preclinical models of depression.<sup>49</sup> Chronic treatment with lithium has been shown to increase synaptic glutamate uptake, and valproic acid dose-dependently increases hippocampal glutamate levels by elevating EAAT1 and EAAT2 levels.<sup>36,37,50</sup> These data further support the hypothesis that excessive glutamate is integral to the pathophysiology that underlies mood disorders. In 2010, Colton and coworkers performed a high-throughput screen to identify small molecules that enhance EAAT2 protein levels in a primary astrocyte cell line.<sup>51</sup> This effort led to the discovery of a series of thiopyridazines, represented by **19**, that provided a range of potencies ( $EC_{50}$ s 500 nM to 3  $\mu$ M) and efficacies (2- to >6-fold) as EAAT2 activators (Figure 10.3).<sup>51</sup> Behavioral data with a targeted EAAT2 activator are eagerly awaited.

### 10.3.3 NMDA Antagonists

NMDA receptors exist as tetrameric complexes comprised of two NR1 and two NR2 subunits, each with multiple splice variants.<sup>14</sup> The glutamate binding site is located on the NR2 subunit, and the obligatory co-agonist glycine binds within NR1.<sup>14</sup> Skolnick and coworkers first demonstrated that chronic antidepressant treatment regulates NMDA receptor expression and function.<sup>8</sup> D-cycloserine, **20**, an antibiotic, showed modest antidepressant and anxiolytic-like properties in rodents, and later was shown to be a partial NMDA receptor antagonist.<sup>52,53</sup> The first generation of potent NMDA receptor antagonists included memantine, **21**, dizocilpine (MK-801), **22**, phencyclidine, **23**, and ketamine, **24** (Figure 10.3). Previous studies have shown that NMDA receptor antagonists are efficacious across multiple preclinical models predictive of antidepressant-like activity, including inescapable stressor, tail suspension and forced-swim-induced immobility tests and in animals exposed to chronic mild stress procedures.<sup>54–60</sup> Clinical studies using the NMDA receptor antagonist ketamine have also demonstrated rapid and robust antidepressant effects in MDD patients after single or repeated dosing regimens.<sup>61–63</sup> Berman *et al.* first reported that IV infusion of ketamine (0.5 mg/kg) resulted in rapid improvements of depressive symptoms within 72 hours in a small group of treatment-resistant MDD patients.<sup>61</sup> In a second double-blind, placebo-controlled crossover study, efficacy with ketamine was observed within 2 hours of infusion and sustained for 1 week in individuals with treatment-resistant MDD.<sup>62</sup> Interestingly, the response rates observed 24 hours after infusion with ketamine were similar to response rates observed after prolonged (6–8 weeks) traditional antidepressant therapy.<sup>64,65</sup> Ketamine infusions have also produced rapid antidepressant effects in patients that are refractory to electroconvulsive therapy, suffering from depression with persistent pain states or alcohol dependence

and in patients with bipolar depression.<sup>66–71</sup> Moreover, suicidal ideations are rapidly decreased in treatment-resistant MDD patients after treatment with ketamine.<sup>72</sup> More recently, Salvatore and coworkers reported that increased anterior cingulate cortical activity and downstream interactions with the amygdala are positively correlated with a rapid antidepressant response to ketamine in drug-free patients with MDD.<sup>73</sup> These findings suggest that pre-treatment levels of anterior cingulate cortical activation may serve as a useful biomarker that identifies patient subgroups who will respond favorably to ketamine's antidepressant effects.<sup>74</sup> Unfortunately, while the sub-anaesthetic doses of ketamine used in these MDD studies appear to present an acceptable level of risk, ketamine and other NMDA receptor antagonists are associated with dose-limiting adverse effects, including psychotomimetic and sedative symptoms, perceptual and cognitive impairments and/or potential abuse liability.<sup>75–76</sup> In addition, ketamine requires an IV route of administration and cannot readily be developed as an orally bioavailable drug for more extensive use in MDD patient populations. These findings have led many in the field to speculate that pharmacologic approaches that either indirectly or more selectively decrease NMDA receptor neurotransmission may alleviate the symptoms of MDD without the adverse effects of direct acting NMDA receptor antagonists.

In order to avoid the liabilities of ketamine, more selective NMDA receptor antagonists, such as those that selectively target the NR2B receptor subtype have been developed and exhibit diminished psychotomimetic effects. Further validation for the inhibition of NMDA receptor function for the treatment of MDD has come from a recent double-blind, placebo-controlled study using the NR2B subunit-selective NMDAR antagonist, CP-101,606, **25**, that produced robust antidepressant efficacy in MDD patients (Figure 10.3).<sup>77</sup> Development of multiple chemotypes of NR2B antagonists is ongoing. Taken together, these preclinical and clinical studies indicate that inhibition of NMDA receptor neurotransmission may represent an exciting and novel approach for the treatment of symptoms associated with MDD, and ketamine and riluzole have been heralded as the prototypes for the next generation of antidepressants and mood stabilizers.

### 10.3.4 AMPA Potentiators

AMPA receptors are comprised of either a homo- or a heteromeric complex of four subunits, denoted Glu1–4, and mediate fast glutamate neurotransmission.<sup>14</sup> As such, AMPA receptors also play critical roles in both learning and memory.<sup>78</sup> AMPA receptor regulation is pronounced in the pathophysiology of mood disorders. Decreased GluR2 and GluR3 have been reported in MDD patients in the prefrontal cortex and decreased GluR1 and GluR2 in BPD patients in the prefrontal cortex and striatum.<sup>79–82</sup> Moreover, classical antidepressants, mood stabilizers and ketamine all stimulate AMPA receptors, increase AMPA receptors surface expression or increase Glu1–4 expression and/or phosphorylation.<sup>83–86</sup> Discovery efforts have focused on developing a new class of therapeutics, AMPA receptor positive allosteric

modulators (PAMs). AMPA PAMs do not activate AMPA receptors, but rather slow the rate of receptor desensitization and/or deactivation in the presence of the orthosteric agonist.<sup>87–88</sup> A diverse array of AMPA receptor PAMs have been disclosed, including cyclothiazide, **26**, CX-516, **27**, LY404187, **28**, and LY392098, **29**, with multiple PAMs displaying antidepressant-like activity in several preclinical models (Figure 10.3).<sup>87,89</sup> In one key study, CX-516 induced a more rapid antidepressant response than fluoxetine,<sup>90</sup> and in another LY392098 was shown to augment standard antidepressants in mouse models, suggesting AMPA receptor PAMs may be beneficial as adjunct therapy in mood disorders.<sup>91</sup> Validation of AMPA receptor PAMs in human clinical trials is eagerly awaited.

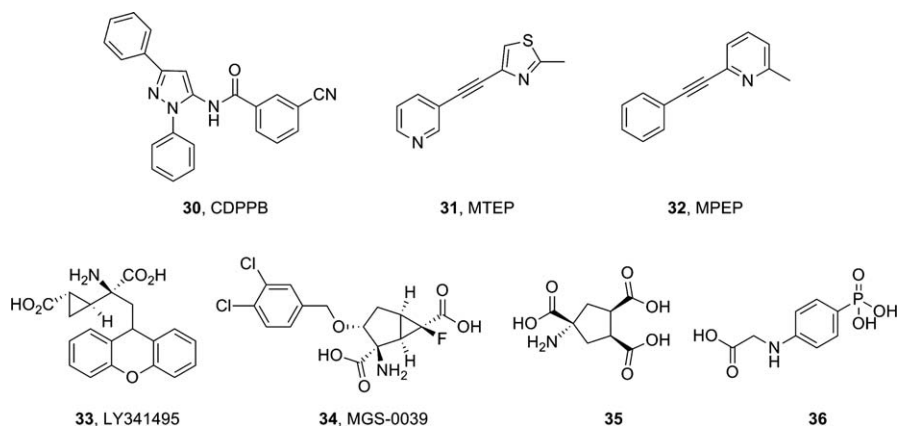
### 10.3.5 Metabotropic Glutamate Receptors

The metabotropic glutamate receptors (mGlu) are members of the GPCR family C, characterized by a large extra-cellular amino-terminal agonist binding domain.<sup>26</sup> To date, eight mGlu have been cloned, sequenced and assigned to three groups (Group I: mGlu1 and mGlu5; Group II: mGlu2 and mGlu3; Group III: mGlu4,6,7,8) based on their sequence homology, pharmacology and coupling to effector mechanisms. Group I mGlu physically and functionally interact with the NMDA receptor to regulate post-synaptic excitability.<sup>26</sup> In recent studies, the metabotropic glutamate receptor subtype 5 (mGlu5) has been shown to function as a close signalling partner with NMDA receptors and may provide key regulation of NMDA receptor functions, including synaptic plasticity in limbic regions thought to be disrupted in individuals with MDD and anxiety disorders.<sup>92–96</sup> For example, mGlu5 can physically interact with NMDA receptors through scaffolding proteins resulting in the enhancement of NMDA receptor currents, as demonstrated by multiple mGlu5 PAMs, such as CDPPB, **30**.<sup>92–95</sup> Moreover, mGlu5 knockout (KO) mice display a decreased NMDA receptor component of evoked excitatory post-synaptic current indicating that mGlu5 is critical for sustaining NMDA receptor neurotransmission.<sup>96</sup> The selective mGlu5 negative allosteric modulator (NAMs) MTEP, **31**, and MPEP, **32**, can also potentiate the behavioral effects of NMDA receptor antagonists in several preclinical models (Figure 10.4).<sup>97–98</sup> These studies suggest that selective inhibition of mGlu5 may provide an important alternative approach for the reduction of NMDA receptor signalling for the treatment of MDD. In support of this hypothesis, Li and coworkers demonstrated that mGlu5 KO mice exhibit antidepressant-like effects, specifically decreased immobility in the forced-swim test that are not resolved by treatment with mGlu5 antagonists.<sup>99</sup> Multiple studies have also reported that MTEP and MPEP have robust antidepressant-like, as well as anxiolytic-like, effects in a number of preclinical models, including tail-suspension, forced swim, punished responding and elevated plus maze tests.<sup>100–107</sup> In contrast to NMDA receptor antagonists, mGlu5 NAMs do not display many of the

dosing-limiting side-effects associated with direct antagonism of NMDA receptors. In summary, these studies provide strong evidence to test the hypothesis that selective antagonism of mGlu5 may result in rapid and robust efficacy for the treatment of symptoms associated with MDD. Currently, both AstraZeneca and Roche have mGlu5 NAMs, AZD2066, AZD2516, RG7090, RO4917523 (structures unknown), in clinical trials for either MDD or treatment-resistant depression.

The Group II mGlu (mGlu2 and mGlu3), modulate glutamatergic transmission by regulating Glu release, which makes them attractive therapeutic targets for treatment of mood disorders.<sup>26,108</sup> In a micro-array study, abnormal mGlu3 expression was noted in suicidal patients with BPD, and an association study in Japan found a correlation with the *GRM3* gene and MDD.<sup>109</sup> Substantial preclinical evidence supports both an antidepressant and anxiolytic role for mGlu2 and mGlu3 agents.<sup>110–111</sup> The mGlu2/mGlu3 antagonists LY341495, **33**, dose-dependently decrease immobility time in animal models of depression, while MGS-0039, **34**, was efficacious in the learned helplessness model of depression<sup>112–113</sup> (Figure 10.4). Further studies have shown that AMPA receptor activation underlies some of the antidepressant activity with these group II mGlu ligands.<sup>114</sup>

Group III mGlu (mGlu7 and mGlu8) are also implicated in the pathophysiology of mood disorders. mGlu7 knockout mice show antidepressant-like effects in preclinical models such as forced swim and tail suspension.<sup>115</sup> Pharmacologically, both the Group II agonist **35** and the mGlu8 agonist **36** elicited significant antidepressant-like effects in the forced-swim test in rats (Figure 10.4).<sup>116</sup> Progress within the Group III mGlu has been slow due to a lack of selective small-molecule tools. Taken together, multiple mGlu have the potential to serve as high-profile molecular targets for the next generation of mood disorder therapeutics.



**Figure 10.4** Structures of the small-molecule mGlu ligands that target the glutamatergic system for the treatment of mood disorders.

## 10.4 Conclusion

Over the last two decades, there has been little success in the development of novel antidepressant therapies that provide a rapid onset of action and efficacy for the depressed mood and anhedonic symptoms observed in patients with mood disorders. Recent preclinical and clinical studies have provided strong data sets implicating dysfunction of the glutamatergic system in the pathophysiology of mood disorders. While preclinical studies of efficacy of agents targeting glutamatergic systems are encouraging, these studies must be considered with caution. Recent findings of robust efficacy of the NMDA receptor antagonist ketamine in treatment of MDD in patients that are refractory to monoamine-based therapies are especially exciting. These studies, coupled with clinical effects of riluzole and other agents that can alter glutamatergic transmission are providing a growing body of evidence that is stimulating focused efforts to develop novel therapeutic approaches for treatment of mood disorders that target different aspects of glutamatergic transmission. If successful, these approaches could provide the first major departure from monoamine-based therapies since their introduction in the 1950s. More importantly, based on the exciting advances with ketamine, it is hoped that new treatments based on regulating transmission through glutamatergic circuits could provide more rapid onset of action and provide efficacy in patients that are resistant to existing treatments.

## Acknowledgements

The authors acknowledge the Department of Pharmacology and NIH for support. Vanderbilt is a member of the MLPCN and houses the Vanderbilt Specialized Chemistry Center.

## References

1. R. C. Kessler, P. Berglund, O. Demler, R. Jin, K. R. Merikangas and E. E. Walters, *Arch. Gen. Psychiatry*, 2005, **62**, 593.
2. A. Fagiolini, D. J. Kupfer, A. Masalehdan, J. A. Scott, P. R. Houck and E. Frank, *Bipolar Disord.*, 2005, **7**, 281.
3. N. Huxley and R. J. Baldessarini, *Bipolar Disord.*, 2007, **9**, 183.
4. M. Tohen, C. A. Zarate Jr, J. Hennen, H. M. Khalsa, S. M. Strakowski, P. Gebre-Medhin, P. Salvatore and R. J. Baldessarini, *Am. J. Psychiatry*, 2003, **160**, 2099.
5. American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, Task Force, Washington, DC, 4th edn, 2000.
6. C. J. Murray and A. D. Lopez, *Science*, 1996, **274**, 740.
7. S. R. Wisniewski, A. J. Rush, C. Bryan, R. Shelton, M. H. Trivedi, S. Marcus, M. M. Husain, S. D. Hollon, M. Fava and STAR\*D Investigators, *J. Nerv. Ment. Dis.*, 2007, **195**, 219.
8. P. Skolnick, P. Popik and R. Trullas, *Trends Pharmacol. Sci.*, 2009, **30**, 563.



9. M. H. Trivedi, A. J. Rush, S. R. Wisniewski, A. A. Nierenberg, D. Warden, L. Ritz, G. Norquist, R. H. Howland, B. Lebowitz, P. J. McGrath, K. Shores-Wilson, M. M. Biggs, G. K. Balasubramani, M. Fava and STAR\*D Study Team, *Am. J. Psychiatry*, 2006, **163**, 28.
10. A. J. Rush, M. H. Trivedi, S. R. Wisniewski, A. A. Nierenberg, J. W. Stewart, D. Warden, G. Niederehe, M. E. Thase, P. W. Lavori, B. D. Lebowitz, P. J. McGrath, J. F. Rosenbaum, H. A. Sackeim, D. J. Kupfer, J. Luther and M. Fava, *Am. J. Psychiatry*, 2006, **163**, 1905.
11. A. A. Nierenberg, M. M. Husain, M. H. Trivedi, M. Fava, D. Warden, S. R. Wisniewski, S. Miyahara and A. J. Rush, *Psychol. Med.*, 2010, **40**, 41.
12. R. C. Kessler, P. Berglund, O. Demler, R. Jin, D. Koretz, K. R. Merikangas, A. J. Rush, E. E. Walters and P. S. Wang, *J. Amer. Med. Assoc.*, 2003, **289**, 3095.
13. R. S. Duman and L. M. Monteggia, *Biol. Psychiatry*, 2006, **59**, 1116.
14. R. Dingledine, K. Borges, D. Bowie and S. F. Traynelis, *Pharmacol. Rev.*, 1999, **51**, 7.
15. H. Mitani, Y. Shirayama, T. Yamada, K. Maeda, C. R. Ashby Jr and R. Kawahara, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2006, **30**, 1155.
16. K. Hashimoto, A. Sawa and M. Iyo, *Biol. Psychiatry*, 2007, **62**, 1310.
17. M. Maes, R. Verkerk, E. Vandoelaeghe, A. Lin and S. Scharpé, *Acta Psychiatr. Scand.*, 1998, **97**, 302.
18. E. Ampuero, F. J. Rubio, R. Falcon, M. Sandoval, G. Diaz-Veliz, R. E. Gonzalez, N. Earle, A. Dagnino-Subiabre, F. Aboitiz, F. Orrego and U. Wyneken, *Neuroscience*, 2010, **169**, 98.
19. L. R. Reznikov, C. A. Grillo, G. G. Piroli, R. K. Pasumarthi, L. P. Reagan and J. Fadel, *Eur. J. Neurosci.*, 2007, **25**, 3109.
20. K. Tokarski, B. Bobula, J. Wabno and G. Hess, *Neuroscience*, 2008, **153**, 789.
21. L. Musazzi, M. Milanese, P. Farisello, S. Zappettini, D. Tardito, V. S. Barbiero, T. Bonifacino, A. Mallei, P. Baldelli, G. Racagni, M. Raiteri, F. Benfenati, G. Bonanno and M. Popoli, *PLoS One*, 2010, **5**, e8566.
22. M. R. Machado-Vieira, H. K. Manji and Z. Zarate, *Neuroscientist*, 2009, **15**(5), 525.
23. M. Erecinska and I. A. Silver, *Prog. Neurobiol.*, 1990, **35**, 245.
24. H. Varoqui, M. K. Schafer, H. Zhu, E. Weihe and J. D. Erickson, *J. Neurosci.*, 2002, **22**, 142.
25. E. Herzog, J. Gilchrist, C. Gras, A. Muzerelle, P. Ravassard, B. Giros, P. Gaspar and S. E. Mestikawy, *Neuroscience*, 2004, **123**, 983.
26. C. M. Niswender and P. J. Conn, *Annu. Rev. Pharmacol. Toxicol.*, 2010, **50**, 295.
27. J. D. Rothstein, L. Jin, M. Dykes-Hoberg and R. W. Kuncel, *Proc. Natl Acad. Sci. USA*, 1993, **90**, 6591–6595.
28. K. Tanaka, K. Watase, T. Manabe, K. Yamada, M. Watanabe, K. Takahashi, H. Iwama, T. Nishikawa, N. Ichihara, T. Kikuchi, S. Okuyama, N. Kawashima, S. Hori, M. Takimoto and K. Wada, *Science*, 1997, **276**, 1699.



29. G. Nowak, R. Trullas, R.T. Layer, P. Skolnick and I. A. Paul, *J. Pharmacol. Exp. Ther.*, 1993, **265**, 1380.
30. I. A. Paul, R. T. Layer, P. Skolnick and G. Nowak, *Eur. J. Pharmacol.*, 1993, **247**, 305.
31. I. A. Paul, G. Nowak, R. T. Layer, P. Popik and P. Skolnick, *J. Pharmacol. Exp. Ther.*, 1994, **269**, 95.
32. G. Nowak, B. Legutko, P. Skolnick and P. Popik, *Eur. J. Pharmacol.*, 1998, **342**, 367.
33. M.-L. Wong, M. A. Smith, J. Licinio, S. Q. Doi, S. R. B Weiss, R. M. Post and P. W. Gold, *Epilepsy Res.*, 1993, **14**, 221.
34. P. Naylor, C. A. Stewart, S. R. Wright, R. C. Pearson and I. C. Reid, *Brain Res. Mol. Brain Res.*, 1996, **35**, 349.
35. P. Svenningsson, E. T. Tzavara, J. M. Witkin, A. A. Fienberg, G. G. Nomikos and P. Greengard, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 3182.
36. S. Nonaka, C. J. Hough and D. M. Chuang, *Proc. Natl Acad. Sci. USA*, 1998, **95**, 2642.
37. K. Kim, S.-G. Lee, T. P. Kegelmann, Z.-Z. Su, S. K. Das, R. Dash, S. Dasgupta, P. M. Barral, M. Hedvat, P. Diaz, J. C. Reed, J. L. Stebbins, M. Pellecchia, D. Sarkar and P. B. Fisher, *J. Cell. Physiol.*, 2011, **226**, 2484.
38. J. Du, N. A. Gray, C. Falke, P. Yuan, S. Szabo and H. K. Manji, *Ann. NY Acad. Sci.*, 2003, **1003**, 378.
39. S. Ahmad, L. J. Fowler and P. S. Whitton, *Arch. Pharmacol.*, 2005, **371**, 1.
40. J. Du, K. Suzuki, Y. Wei, Y. Wang, R. Blumenthal, Z. Chen, C. Falke, C. A. Zarate, Jr and H. K. Manji, *Neuropsychopharmacology*, 2007, **32**, 793.
41. M. E. Wolf, X. Sun, S. Mangiavacchi and S. Z. Chao, *Neuropharmacology*, 2004, **47**, 61.
42. C. A. Zarate, Jr, J. L. Payne, J. Quiroz, J. Sporn, K. K. Denicoff, D. Luckenbaugh, D. S. Charney and H. K. Manji, *Am. J. Psychiatry*, 2004, **161**, 171.
43. C. A. Zarate, Jr, J. A. Quiroz, J. B. Singh, K. D. Denicoff, G. De Jesus, D. A. Luckenbaugh, D. S. Charney and H. K. Manji Biol, *Psychiatry*, 2005, **57**, 430.
44. G. Sanacora, S. F. Kendell, Y. Levin, A. A. Simen, L. R. Fenton, V. Coric and J. H. Krystal, *Biol. Psychiatry*, 2007, **61**, 822.
45. M. E. Frizzo, L. P. Dall'Onder, K. B. Dalcin and D. O. Souza, *Cell Mol. Neurobiol.*, 2004, **24**, 123.
46. R. H. Brown Jr, *N. Engl. J. Med.*, 2005, **352**, 1376.
47. T. M. Miller and D. W. Cleveland, *Science*, 2005, **307**, 361.
48. J. D. Rothstein, S. Patel, M. R. Regan, C. Haenggeli, Y. H. Huang, D. E. Bergles, L. Jin, M. Dykes Hoberg, S. Vidensky, D. S. Chung, S. V. Toan, L. I. Bruijn, Z.-Z. Su, P. Gupta and P. B. Fisher, *Nature*, 2005, **433**, 73.
49. Y. S. Mineur, M. R. Picciotto and G. Sanacora, *Biol. Psychiatry*, 2006, **61**, 250.
50. B. Hassel, E. G. Iversen, L. Gjerstad and E. Taubøll, *J. Neurochem.*, 2001, **77**(5), 1285.

51. C. K. Colton, Q. Kong, L. Lai, M. X. Zhu, K. I. Seyb, G. D. Cuny, J. Xian, M. A. Glicksman and C. L. Lin, *J. Biomol. Screening*, 2010, **15**(6), 653.
52. G. Crane, *Am. J. Psychiatry*, 1959, **115**, 1025.
53. B. N. M. van Berckel, C. Lipsch, S. Timp, C. Gispen-de Wied, H. Wynne, J. M. van Ree and R. S. Kahn, *Neuropsychopharmacology*, 1997, **16**, 317.
54. M. Papp and E. Moryl, *Eur. J. Pharmacol.*, 1994, **263**(1–2), 1.
55. E. Przeglasiński, E. Tatarczyńska, A. Dereń-Wesołek and E. Chojnacka-Wojcik, *Neuropharmacology*, 1997, **36**(1), 31.
56. A. Yilmaz, D. Schulz, A. Aksoy and R. Canbeyli, *Pharmacol. Biochem. Behav.*, 2002, **71**(1–2), 341.
57. L. S. Garcia, C. M. Comim, S. S. Valvassori, G. Z. L. Stertz, F. Kapczinski, E. C. Gavioli and J. Quevedo, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2009, **33**(3), 450.
58. S. Maeng, C. A. Zarate Jr, J. Du, R. J. Schloesser, J. McCammon, G. Chen and H. K. Manji, *Biol. Psychiatry*, 2008, **63**(4), 349.
59. F. C. da Silva, M. do Carmo de Oliveira Cito, M. I. da Silva, B. A. Moura, N. de Aquino, M. R. Neto, M. L. Feitosa, R. de Castro Chaves, D. S. Macedo, S. M. de Vasconcelos, M. M. de França Fonteles and F. C. de Sousa, *Brain Res. Bull.*, 2010, **83**(1–2), 9.
60. L. S. Garcia, C. M. Comim, S. S. Valvassori, G. Z. Réus, L. Stertz, G. Flavio, E. C. Gavioli and J. Quevedo, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2009, **33**, 450.
61. R. M. Berman, A. Cappiello, A. Anand, D. A. Oren, G. R. Heninger, D. S. Charney and J. H. Krystal, *Biol. Psychiatry*, 2000, **47**(4), 351.
62. C. A. Zarate Jr, J. B. Singh, P. J. Carlson, N. E. Brutsche, R. Ameli, D. A. Luckenbaugh, D. S. Charney and H. K. Manji, *Arch. Gen. Psychiatry*, 2006, **63**(8), 856.
63. M. Rot, K. A. Collins, J. W. Murrough, A. M. Perez, D. L. Reich, D. S. Charney and S. J. Mathew, *Biol. Psychiatry*, 2010, **67**(2), 139.
64. A. R. Entsuah, H. Huang and M. E. Thase, *J. Clin. Psychiatry*, 2001, **62**(11), 869.
65. M. E. Thase, B. R. Haight, N. Richard, C. B. Rockett, M. Mitton, J. G. Modell, S. VanMeter, A. E. Harriett and Y. Wang, *J. Clin. Psychiatry*, 2005, **66**(8), 974.
66. L. Ibrahim, N. Diazgranados, D. A. Luckenbaugh, R. Machado-Vieira, J. Baumann, A. G. Mallinger and C. A. Zarate Jr, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2011, **35**(4), 1155–1159.
67. G. E. Correll and G. E. Futter, *Pain Med.*, 2006, **7**(1), 92.
68. L. Stefanczyk-Sapieha, D. Oneschuk and M. Demas, *J. Palliat. Med.*, 2008, **11**(9), 1268.
69. L. E. Phelps, N. Brutsche, J. R. Moral, D. A. Luckenbaugh, H. K. Manji and C. A. Zarate Jr, *Biol. Psychiatry*, 2009, **65**(2), 181.
70. D. Thangathurai and M. Mogos, *J. Palliat. Med.*, 2011, **14**(4), 389.

71. N. DiazGranados, L. Ibrahim, N. E. Brutsche, A. Newberg, P. Kronstein, S. Khalife, W. A. Kammerer, Z. Quezado, D. A. Luckenbaugh, G. Salvatore, R. Machado-Vieira, H. K. Manji and C. A. Zarate Jr, *Arch. Gen. Psychiatry*, 2010, **67**(8), 793–802.
72. N. DiazGranados, L. A. Ibrahim, N. E. Brutsche, R. Ameli, I. D. Henter, D. A. Luckenbaugh, R. Machado-Vieira and C. A. Zarate, *J. Clin. Psychiatry*, 2010, **71**(12), 1605.
73. G. Salvatore, B. R. Cornwell, F. Sambataro, D. Latov, V. Colon-Rosario, F. Carver, T. Holroyd, N. DiazGranados, R. Machado-Vieira, C. Grillon, W. C. Drevets and C. A. Zarate Jr, *Neuropsychopharmacology*, 2010, **35**, 1415.
74. G. Salvatore, B. R. Cornwell, V. Colon-Rosario, R. Coppola, C. Grillon, C. A. Zarate Jr and H. K. Manji, *Biol. Psychiatry*, 2009, **65**(4), 289.
75. J. H. Krystal, L. P. Karper, J. P. Seibyl, G. K. Freeman, R. Delaney, J. D. Bremner, G. R. Heninger, M. B. Bowers Jr and D. S. Charney, *Arch. Gen. Psychiatry*, 1994, **51**(3), 199.
76. P. Dillon, J. Copeland and K. Jansen, *Drug Alcohol Depend.*, 2003, **69**(1), 23.
77. S. H. Preskorn, B. Baker, S. Kolluri, F. S. Menniti, M. Krams and J. W. Landen, *J. Clin. Psychopharmacol.*, 2008, **28**(6), 631.
78. M. D. Black, *Psychopharmacology*, 2005, **179**, 154.
79. K. Hashimoto, A. Sawa and M. Iyo, *Biol. Psychiatry*, 2007, **62**, 1310.
80. E. Scarr, G. Pavey, S. Sundram, A. MacKinnon and B. Dean, *Bipolar Disord.*, 2003, **5**, 257.
81. M. Beneyto and J. H. Meador-Woodruff, *Synapse*, 2006, **60**, 585.
82. J. H. Meador-Woodruff, A. J. Hogg Jr and R. E. Smith, *Brain Res. Bull.*, 2001, **55**, 631.
83. J. Du, N. A. Gray, C. A. Falke, W. Chen, P. Yuan, S. T. Szabo, H. Einat and H. K. Manji, *J. Neurosci.*, 2004, **24**, 6578.
84. J. Du, K. Suzuki, Y. Wei, Y. Wang, R. Blumenthal, Z. Chen, C. Falke, C. A. Zarate, Jr and H. K. Manji, *Neuropsychopharmacology*, 2007, **32**, 793.
85. R. Martinez-Turrillas, J. Del Rio and D. Frechilla, *Biochem. Biophys. Res. Commun.*, 2007, **353**, 750.
86. Z. Bhagwagar and G. M. Goodwin, *Expert Opin. Pharmacother.*, 2005, **6**, 1401.
87. M. D. Black, *Psychopharmacology*, 2005, **179**, 154.
88. D. Bleakman and D. Lodge, *Neuropharmacology*, 1998, **37**, 1187.
89. P. Miu, K. R. Jarvie, V. Radhakrishnan, M. R. Gates, A. Ogden, P. L. Ornstein, H. Zarrinmayeh, K. Ho, D. Peters and J. Grabbell, *Neuropharmacology*, 2001, **40**, 976.
90. R. J. Knapp, R. Goldenberg, C. Shuck, A. Cecil, J. Watkins, C. Miller, G. Crites and E. Malatynska, *Eur. J. Pharmacol.*, 2002, **440**, 27.
91. X. Li, J. M. Witkin, A. B. Need and P. Skolnick, *Cell Mol. Neurobiol.*, 2003, **23**, 419.

92. J. E. Ayala, Y. Chen, J. L. Banko, D. J. Sheffler, R. Williams, A. N. Telk, N. L. Watson, Z. Xiang, Y. Zhang, P. J. Jones, C. W. Lindsley, M. F. Olive and P. J. Conn, *Neuropsychopharmacology*, 2009, **34**(9), 2057.
93. G. Mannaioni, M. J. Marino, O. Valenti, S. F. Traynelis and P. J. Conn, *J. Neurosci.*, 2001, **21**(16), 5925.
94. M. J. Marino and P. J. Conn, *Curr. Opin. Pharmacol.*, 2006, **6**(1), 98.
95. S. Alagarsamy, M. J. Marino, S. T. Rouse, R. W. Gereau 4th, S. F. Heinemann and P. J. Conn, *Nat. Neurosci.*, 1999, **2**(3), 234.
96. Z. Jia, Y. Lu, J. Henderson, F. Taverna, C. Romano, W. Abramow-Newerly, J. M. Wojtowicz and J. Roder, *Learn Mem*, 1998, **5**(4-5), 331.
97. G. G. Kinney, M. Burno, U. C. Campbell, L. M. Hernandez, D. Rodriguez, L. J. Bristow and P. J. Conn, *J. Pharmacol. Exp. Ther.*, 2003, **306**(1), 116.
98. U. C. Campbell, K. Lalwani, L. Hernandez, G. G. Kinney, P. J. Conn and L. J. Bristow, *Psychopharmacology*, 2004, **175**(3), 310.
99. X. Li, A. B. Need, M. Baez and J. M. Witkin, *J. Pharmacol. Exp. Ther.*, 2006, **319**(1), 254.
100. A. Pilc, A. Kłodzińska, P. Brański, G. Nowak, A. Pałucha, B. Szewczyk, E. Tatarczyńska, E. Chojnacka-Wójcik and J. M. Wierońska, *Neuropharmacology*, 2002, **43**(2), 181.
101. E. Tatarczyńska, A. Kłodzińska, E. Chojnacka-Wójcik, A. Palucha, F. Gasparini, R. Kuhn and A. Pilc, *Br. J. Pharmacol.*, 2001, **132**(7), 1423.
102. A. Pałucha, P. Brański, B. Szewczyk, J. M. Wierońska, K. Kłak and A. Pilc, *Pharmacol. Biochem. Behav.*, 2005, **81**(4), 901.
103. M. Pietraszek, I. Sukhanov, P. Maciejak, J. Szyndler, A. Gravius, A. Wisłowska, A. Płażnik, A.Y. Beshpalov and W. Danysz, *Eur. J. Pharmacol.*, 2005, **514**(1), 25.
104. C. S. Busse, J. Brodtkin, D. Tattersall, J. J. Anderson, N. Warren, L. Tehrani, L. J. Bristow, M. A. Varney and N. D. P. Cosford, *Neuropsychopharmacology*, 2004, **29**, 1971.
105. J. Brodtkin, C. Busse, S. J. Sukoff and M. A. Varney, *Pharmacol. Biochem. Behav.*, 2002, **73**, 359.
106. B. Schulz, M. Fendt, F. Gasparini, K. Lingenhohl, R. Kuhn and M. Koch, *Neuropharmacology*, 2001, **41**, 1.
107. A. L. Rodriguez, M. D. Grier, C. K. Jones, E. J. Herman, A. S. Kane, R. L. Smith, R. Williams, Y. Zhou, J. E. Marlo, E. L. Days, T. N. Blatt, S. Jadhav, U. Menon, P. N. Vinson, J. M. Rook, S. R. Stauffer, C. M. Niswender, C. W. Lindsley, C. D. Weaver and P. J. Conn, *Mol. Pharmacol.*, 2010, **78**, 1105.
108. J. A. Morrow, R. Gilfillan and S. A. Neale, in *Drug Discovery for Psychiatric Disorders*, ed. Z. Rankovic, M. Bingham, R. Hargreaves and E. Nestler, The Royal Society of Chemistry, Cambridge, 2012, p 56.
109. T. Tsunoka, T. Kishi, M. Ikeda, T. Kitajima, Y. Yamanouchi, Y. Kinoshita, K. Kawashima, T. Okochi, T. Okumura, T. Inada, N. Ozaki and N. Iwata, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2009, **33**, 875.

110. A. Palucha and A. Pilc, *Pharmacol. Ther.*, 2007, **115**, 116.
111. J. M. Witkin, G. J. Marek, B. G. Johnson and D. D. Schoepp, *CNS Neurol. Disord. Drug Targets*, 2007, **6**, 87.
112. S. Chaki, R. Yoshikawa, S. Hirota, T. Shimazaki, M. Maeda, N. Kawashima, T. Yoshimizu, A. Yasuhara, K. Sakagami and S. Okuyama, *Neuropharmacology*, 2004, **46**, 457.
113. T. Yoshimizu, T. Shimazaki, A. Ito and S. Chaki, *Psychopharmacology*, 2006, **186**, 587.
114. J. Karasawa, T. Shimazaki, N. Kawashima and S. Chaki, *Brain Res.*, 2005, **1042**, 92.
115. J. F. Cryan, P. H. Kelly, H. C. Neijt, G. Sansig, P. J. Flor and H. van Der Putten, *Eur. J. Neurosci.*, 2003, **17**, 2409.
116. F. Gasparini, V. Bruno, G. Battaglia, S. Lukic, T. Leonhardt, W. Inderbitzin, D. Laurie, B. Sommer, M. A. Varney and S. D. Hess, *J. Pharmacol Exp. Ther.*, 1999, **289**, 1678.

## CHAPTER 11

# *Modulation of HPA Axis Function for Treatment of Mood Disorders*

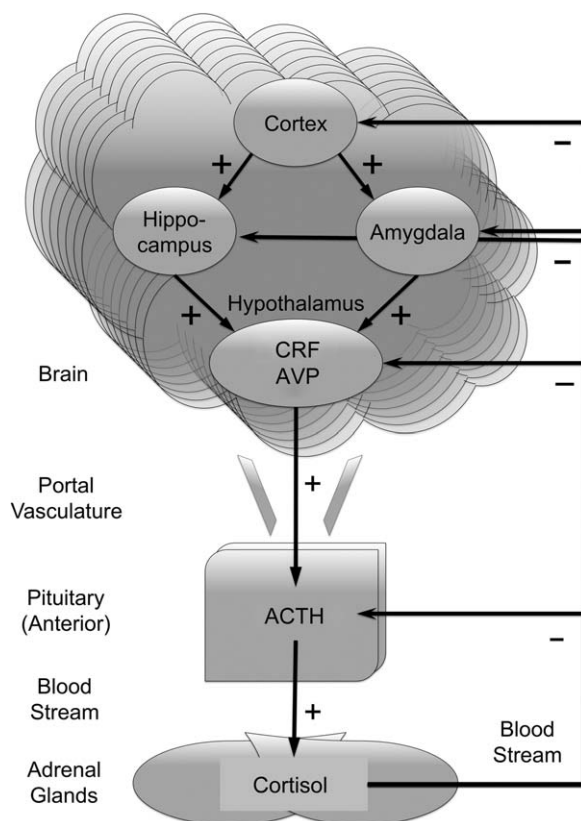
SHAUN P. BROTHERS, CLAES WAHLESTEDT  
AND CHARLES B. NEMEROFF\*

Department of Psychiatry and Behavioral Sciences, University of Miami  
Miller School of Medicine, 1120 Northwest 14 Street, Suite 1455 Miami,  
Florida 33136

\*Email: cnemeroff@med.miami.edu

### 11.1 The Hypothalamic-Pituitary-Adrenal (HPA) Axis

The hypothalamic-pituitary-adrenal (HPA) axis is the fundamental regulator of stress in mammals. Paraventricular neurons, which receive inputs from the hippocampus and other brain areas, release corticotropin-releasing factor (CRF or CRH) from nerve terminals in the median eminence of the hypothalamus. CRF, together with arginine vasopressin (AVP), travels through the portal vasculature to the anterior pituitary to stimulate the synthesis and release of adrenocorticotrophic hormone (ACTH) into the bloodstream. ACTH in turn primarily stimulates cortisol release from the adrenal cortex. Cortisol feeds back to negatively regulate the release of CRF, AVP and ACTH *via* glucocorticoid receptors in the hypothalamus and pituitary and mineralocorticoid receptors in the hippocampus, thus completing the regulatory loop (Figure 11.1).



**Figure 11.1** Hypothalamic-Pituitary-Adrenal axis. Neurotransmitters in the brain coordinate the response from stressful stimuli, causing a release of CRF into the portal vasculature. CRF stimulates the release of ACTH from anterior pituitary corticotrope cells into the general circulation where it travels to the adrenal glands to stimulate cortisol production and systemic release. Cortisol feeds back to the pituitary and brain to negatively regulate CRF and ACTH release. AVP potentiates the effects of CRF on ACTH release.

CRF, first isolated in 1981 by Vale *et al.*,<sup>1</sup> is released in response to stress not only in the hypothalamus but in other CNS regions as well, including the central amygdala (CeA). Although hypothalamic CRF release occurs in response to all types of stress, the CeA-CRF release is believed to mediate much of the emotional component to stress. CRF has far-reaching effects in the body ranging from alterations in metabolic state, sympathetic output, emotional state, appetite and reproductive status, among numerous others.<sup>2-5</sup> Indeed, a great deal of evidence suggests that CRF coordinates the endocrine, autonomic, immune and behavioural responses to stress. There are also significant differences in adaptations to acute and chronic stress, with chronic stress being associated with a number of health consequences such as heart disease, reproductive dysfunction and indeed mood disorders.<sup>6</sup>



## 11.2 The HPA Axis in Mood Disorders

The hypothesis that components of the hypothalamic-pituitary-adrenal (HPA) axis play a role in mood disorders has been discussed for half a century or longer.<sup>7,8</sup> One of the notable early studies was published in 1966; D. J. McClure demonstrated increased cortisol levels in the urine of depressed patients. Results of a number of later studies confirmed and extended these findings by documenting increased cortisol concentrations in blood, urine and cerebrospinal fluid (CSF) in depressed patients. This hypercortisolemia generally occurs during (or immediately preceding) the onset of a mood disorder episode such as major depressive disorder (MDD). Hypercortisolemia is considered a state marker rather than a trait marker of depression,<sup>9</sup> although multiple studies showing its lack of specificity have rendered it a poor biomarker for mood disorders.<sup>10–13</sup>

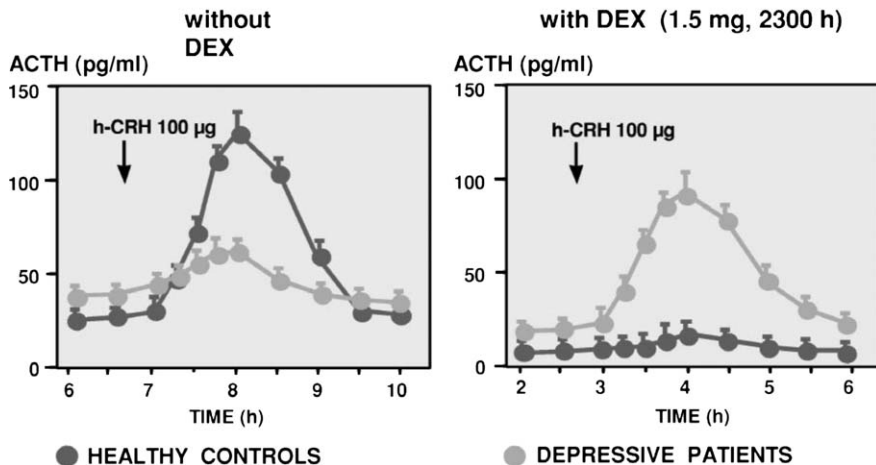
Cortisol is not the only HPA axis hormone to be dysregulated in stress or depression. CRF, ACTH and AVP are also dysregulated in mood disorders.<sup>14</sup> In 1984, Nemeroff *et al.* reported an increase in CRF concentrations in CSF from untreated MDD patients,<sup>14</sup> which has been confirmed in a number of studies.<sup>15,16</sup> CRF concentrations are also increased in the brains of depressed patients.<sup>2</sup> Because CRF signals are transduced *via* a G Protein Coupled Receptor, which are notorious for rapid and long-lasting down-regulation and desensitization, CRF receptor expression at the cellular surface actually decreases in response to an abundance of CRF agonist activity as observed in *post mortem* brain tissue from suicide victims.<sup>17,18</sup> We hypothesize that down-regulation of CRF receptors is deleterious to the disease pathophysiology because CRF negative feedback regulation likely occurs in the brain. Without feedback inhibition, there may be an inability to shut off the HPA axis, including CRF over-production, though this has yet to be directly tested. Indeed, CRF over-expression in certain brain regions such as the central amygdala (CeA) is associated with behavioural anxiety in rodents.<sup>19</sup>

Similar to baseline plasma cortisol, CSF CRF is not a reliable biomarker for mood disorders, though levels are often elevated in depressed patients.<sup>20</sup> The CRF stimulation test is a more accurate measure of HPA axis activity than CSF CRF concentrations in part because the latter represent contributions of both hypothalamic and extra-hypothalamic circuits. In the CRF stimulation test, a bolus intravenous dose of CRF is administered, causing ACTH release from the pituitary and the resulting ACTH (or beta-endorphin) blood concentrations are measured. In normal patients, ACTH increases predictably. However, in depressed patients the ACTH response is stereotypically blunted due to either CRF-mediated down-regulation of CRF receptors or hypercortisolemia.<sup>21–23</sup>

Another even more sensitive test of HPA axis activity is accomplished by administering dexamethasone (DEX) the evening before CRF (Figure 11.2), termed the DEX-CRF test.<sup>24,25</sup>

In normal volunteers without MDD, DEX suppresses ACTH and cortisol secretion *via* feedback inhibition at the pituitary. In patients with MDD, feedback inhibition is blunted by the presence of already high levels of cortisol,

**BLUNTED ACTH RESPONSE TO h-CRH IN DEPRESSIVES IS  
PARADOXICALLY ENHANCED AFTER DEX PRETREATMENT**



**Figure 11.2** Patients with depression usually show a blunted ACTH response to CRF, which is believed to be secondary to desensitized CRF1 receptors (left). After pre-treatment with a low dose of dexamethasone, healthy controls do not show a substantial ACTH response, whereas the ACTH response of patients with depression is comparable to the response of healthy controls not pre-treated with dexamethasone (right). Reprinted from F. Holsboer, *J. Affective Disord.*, 2001, **62**, 77–91, with permission from Elsevier.<sup>25</sup>

little additional suppression takes place and ACTH levels remain high. When CRF is additionally administered to healthy patients, there will be little or no increase in ACTH due to the very robust suppression of ACTH secretion by DEX. However, in MDD patients, due to the dysregulation in the systems described above, the inability of DEX to suppress ACTH as well as with direct stimulation of ACTH release by CRF, circulating levels of ACTH (and cortisol) increase.<sup>24,25</sup> The sensitivity of this DEX-CRF test can predict 9 out of 10 MDD patients correctly, and can even be used to identify asymptomatic or in remission MDD patients who continue to exhibit HPA axis dysfunction and who are therefore at risk for relapse.<sup>26</sup>

### 11.3 Non-mood-related Effects of HPA-Axis Hyperactivity

HPA axis hyperactivity can be severely deleterious on aspects of health aside from the impact on mood disorders. Although we will not review this literature because it is not a focus of this chapter, such adverse effects often additionally complicate mood disorders. Depressed patients suffer higher rates of cardiovascular disease, due in part to dysregulation of the HPA axis.<sup>27</sup> Indeed chronic mood disorders are as threatening to the cardiovascular system as fatty diets or

cigarette smoking; the prevalence of depression in patients with cardiovascular disease can be as high as 1 in 3.<sup>28</sup> It is therefore of major importance that when considering the positive effects of HPA axis modulators on mood disorders one also considers the potential for ameliorating the systemic effects of depression including heart disease, obesity, osteoporosis and immune system dysfunction.

## **11.4 Modulation of the HPA Axis as a Therapeutic Strategy to Treat Mood Disorders**

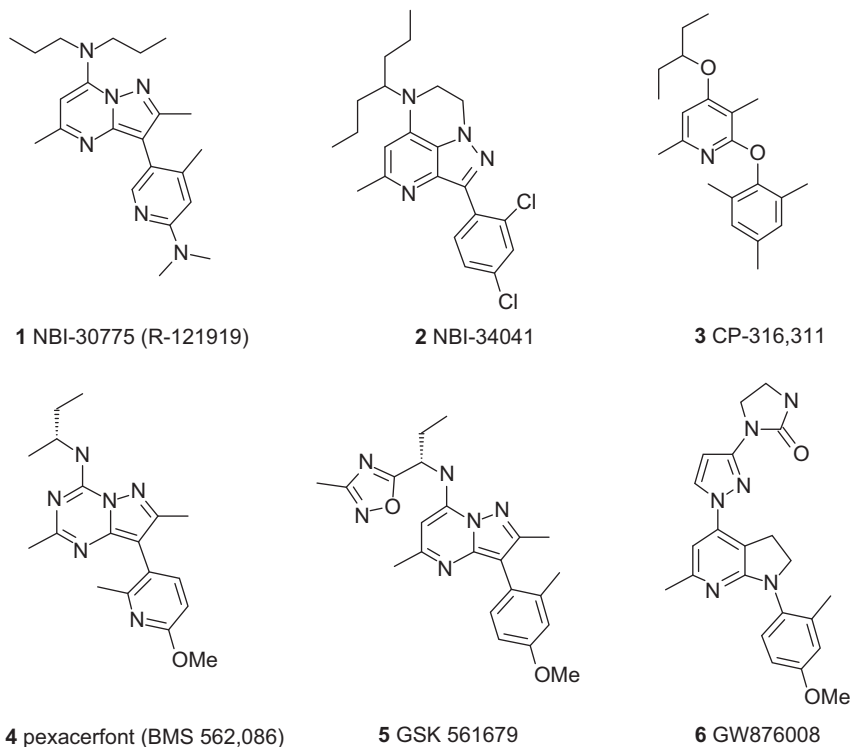
Based upon the voluminous data linking HPA axis hyperactivity to mood disorders, it is plausible that there must be modulation points along the axis where a properly designed therapeutic intervention could positively impact mood disorders with the premise that CRF/ACTH/cortisol and their receptors are indeed pathologically involved in the neurobiology of depression. Some of the most pursued targets in recent years have been the CRF receptors.

CRF G protein coupled receptors have two isoforms, the CRF1 and CRF2 receptors. The CRF1 receptor is predominantly located in the CNS, although it is found outside the brain in the retina, pituitary, spleen, pancreatic islets and in testicular and ovarian cells, and is thought to control HPA axis activity.<sup>2,29</sup> The CRF2 receptor has three spliciforms (denoted 2a, 2b and 2c), a higher affinity for urocortins than CRF and is found both centrally and peripherally.<sup>29</sup> CRF2 receptors are generally thought to mediate some of the peripheral effects of stress. When considering a modulator, it is interesting to speculate on both receptor subtypes but, in actuality, virtually all of the therapeutic development work has focused on the CRF1 receptor.

Antagonism of the CRF1 receptor may decrease basal and stress induced increases in HPA axis tone, reduce the health-related side-effects and, as noted above, perhaps positively impact mood disorder treatment outcomes. The most convincing data supporting a use for CRF1 receptor antagonists come from animal models. For example, CRF1 receptor knockout mice have much reduced ACTH, stress-induced glucocorticoid secretion and reduced anxiety.<sup>30–32</sup> There have been several attempts to develop clinically useful CRF1 receptor antagonists to treat mood disorders, with varying levels of success. It is of paramount importance to note that in terms of mood and anxiety symptoms, CRF1 receptor antagonists are believed to act at extra-hypothalamic/extra-pituitary sites. This has been well documented in preclinical studies.<sup>33</sup> In fact, CRF1 receptor antagonists exhibit anxiolytic and antidepressant effects in hypophysectomized animals.<sup>34</sup>

## **11.5 CRF1 Receptor Antagonists in Mood and Anxiety Disorders**

The first small clinical trial (n = 20) of a CRF1 receptor antagonist for the treatment of mood disorders was published in 2000.<sup>35</sup> In this open-label trial,

**Figure 11.3**

doses of NBI-30775 (R-121919), **1**, (Figure 11.3) were escalated up to 80 mg and produced an antidepressant effect equivalent to the SSRI paroxetine. There was no decrease in basal plasma ACTH or cortisol levels, an effect confirmed in all subsequent studies of CRF1 receptor antagonists. This fact lessened concern that CRF antagonists may produce an Addisonian-like state of adrenal insufficiency.

The same group that tested NBI-30775 conducted a placebo-controlled, double-blind, randomized trial with NBI-34041, **2**, (Figure 11.3, not to be confused with NBI-30775), another CRF1 receptor antagonist.<sup>36</sup> In this study of 24 healthy male volunteers without history of mood disorder, there was no effect on basal plasma ACTH or cortisol concentrations. However, there was a decrease in stimulated ACTH and cortisol levels when patients were administered the Trier social stress test (TSSS), a validated human stress paradigm. Again, the small size of this study and the fact that the subjects were healthy limits the conclusions that can be drawn with regard to the therapeutic potential of this drug in treating mood disorders.

More than 20 clinical trials have been conducted or are currently underway on the effects of CRF1 receptor antagonists on mood and anxiety disorders.

Many of these better-powered clinical trials have been somewhat disappointing by revealing a lack of efficacy for CRF1 receptor antagonists in patients with MDD. In a 6-week randomized, placebo-controlled trial of CP-316,311, **3** (Pfizer) was compared to placebo and sertraline in 128 patients. There was no significant antidepressant effect of the CRF1 antagonist compared to placebo in patients with major depression, although sertraline did have an effect.<sup>37</sup> In the largest study to date, of 260 patients, Coric *et al.* conducted an 8-week multi-centre, randomized, double-blind, placebo-controlled clinical trial with pexacerfont, **4** (BMS-562,086), for generalized anxiety disorder; no significant anxiolytic effect was observed compared to placebo, although the comparator in the study, escitalopram, was efficacious.<sup>38</sup>

It is especially important to note that these studies have not used narrowly defined patient subtypes such as manic, psychotic, anxious or atypical depressive but instead have utilized the broader MDD inclusion criterion. Nor have clinical trials focused on the successful effects of CRF1 receptor antagonists in improving Trier social stress test outcomes; the National Institute of Alcohol Abuse and Alcoholism is currently recruiting for a phase II trial of the CRF1 receptor antagonist, GSK 561679, **5** (GlaxoSmithKline) (Figure 11.3), in stress-induced alcohol craving in women (clinical trial #NCT01187511) and are using the TSSS as an endpoint measure. Glaxo-SmithKline (GSK) has sponsored or collaborated on approximately 15 phase I and II trials with CRF1 receptor antagonists, GW876008, **6**, and/or GSK 561679. There is little currently in the peer-reviewed literature concerning the outcomes of these trials. There are currently no publications of GSK 561679. The only published trial of GW876008 was on the effects of GW876008 on 14 females with irritable bowel syndrome (IBS) as compared to 17 control volunteers. This rigorously designed study sought to determine whether a centrally acting CRF1 receptor antagonist could ameliorate the stress-related emotional–arousal circuit during expectation of abdominal pain as a marker for anxiety-related disorders. Indeed, the authors found a significant reduction in extra-hypothalamic/extra-HPA neural activity with drug *versus* placebo during the expectation of pain.<sup>39</sup> The authors also found no significant change in emotionality.

Considering the fact that there is overwhelming evidence that CRF is hypersecreted in depressed patients with a history of childhood abuse and neglect, a clinical trial of CRF1 receptor antagonists in this discrete subset of patients with major depression would be of considerable interest.<sup>40,41</sup> Moreover, studies have documented elevated CSF CRF concentrations in post-traumatic stress disorder (PTSD).<sup>42</sup> Finally, polymorphisms of the CRF1 receptor have been shown to influence vulnerability for depression and alcoholism.<sup>43–45</sup> It would therefore be of interest to segregate CRF1 receptor antagonist clinical trial outcomes for genotype specific effects in treatment response. Further, taken together with the polymorphism data, there is some evidence that these compounds will be useful in treating alcoholism, which is often highly co-morbid with mood disorders.<sup>46,47</sup>

## 11.6 CRF2 Receptor Antagonists in the Treatment of Mood Disorders

CRF2 receptor knockout mice have been reported to be hypersensitive to stressors,<sup>48,49</sup> though in contrast, Kishimoto found that CRF2 receptor knockout mice displayed an anxiolytic phenotype.<sup>50</sup> These differences may, in part, be attributable to genetic differences between the murine lines used in each of these studies.<sup>51,52</sup> Subsequent to these knockout experiments in 2000, there have been a number of studies suggesting a contribution of the CRF2 receptor to HPA axis regulation and mood disorders. There is evidence that the CRF2 receptor helps to mediate the duration of the HPA response to stress.<sup>48,49,52</sup> By blocking CRF2 receptors in humans, the HPA axis may be more quickly turned off after a stressful event, though the consequences of early HPA axis activity attenuation in stressful situations is unknown.

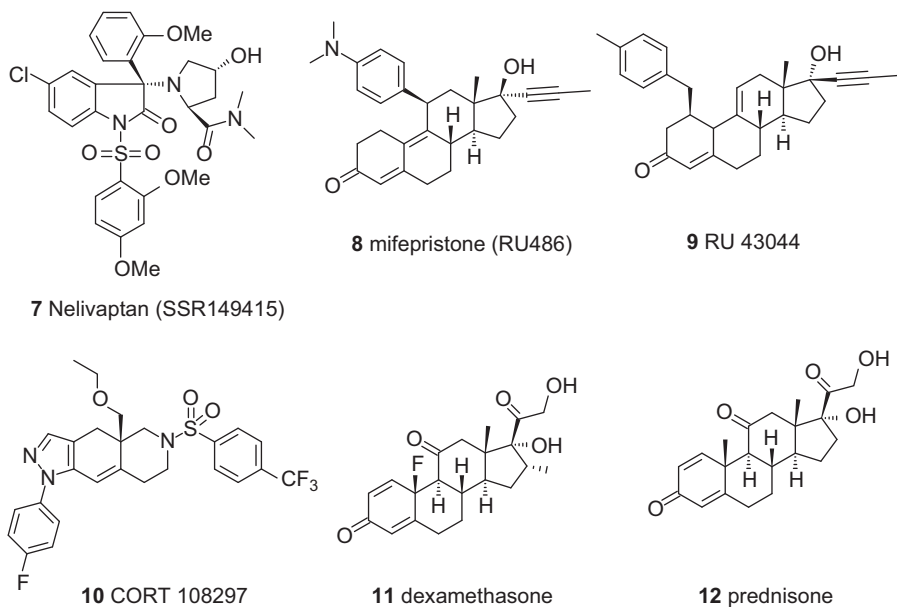
All selective CRF2 receptor agonists and antagonists developed to date, to our knowledge, are peptide based, thus limiting their therapeutic utility. The best of the receptor antagonists are antisauvagine-30, astressin-2B and K41498, which are all peptide based. Neurogen, now Ligand Pharmaceuticals, was purported to have developed a CRF2 receptor antagonist, but little information is publically available on this compound, and the development program was apparently discontinued in 1998.

## 11.7 CRF Binding Protein

CRF binding protein (CRF-BP), a protein that binds to and stabilizes circulating and CNS CRF peptide,<sup>53,54</sup> has received little attention as a novel target for drug development in mood disorders, though it may indeed be an interesting one. In a study of *post mortem* brain tissue CRF-BP mRNA expression was decreased in bipolar and schizophrenia patients.<sup>55</sup> Similarly, in a comparison of patients culled from the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) study,<sup>56</sup> it was found that a single SNP in the gene coding for the CRF-BP was significantly associated with HPA axis hyperactivity and GR resistance, and a poor response to treatment especially in anxious depression.<sup>57</sup> Additionally, there is some evidence that by increasing CRF-BP the amount of bioavailable CRF can be reduced and thus act as a natural brake to HPA axis overdrive.<sup>55,58</sup> Administration of CRF-BP or CRF-BP genetic up-regulation or microRNA targeting might increase systemic CRF-BP levels and reduce CRF hyperactivity. Finally, the EST BF589926 has the potential to be an antisense RNA regulator of CRF-BP.<sup>59</sup> It is currently unclear whether any of these methods represent a viable therapeutic strategy.

## 11.8 Arginine Vasopressin 1b Receptors

Arginine vasopressin (AVP) performs a number of physiological functions, and most notably acts in the periphery to regulate blood osmolarity *via* the AVP 2

**Figure 11.4**

receptor (V2R). However, AVP and AVP receptor activation produce a number of CNS and peripheral effects and have been postulated to play a role in mood disorders.<sup>60</sup> There have also been genetic linkage studies suggesting that early life stress can permanently alter epigenetic modifications at the AVP gene and this effect was reversed by the AVP1b receptor antagonist SSR149415 **7** (nelivaptan) (Figure 11.4).<sup>61</sup> Because AVP is released from the hypothalamus and acts in the anterior pituitary at AVP1b receptors to potentiate the effects of CRF on ACTH release, it is also a potential modulation point in the treatment of mood disorders. This is not a new idea however.<sup>62,63</sup>

SSR149415 is a potent and selective AVP1b receptor antagonist that has been proposed as a novel antidepressant.<sup>63</sup> SSR149415 has been tested in clinical trials for the treatment of major depression. However, a press release in 2008 by Sanofi-Aventis stated that SSR149415 trials would be discontinued, though no reason was given. Considerable interest remains surrounding the development of an AVP 1b receptor antagonist for depression. Such a compound may act synergistically when co-administered with a CRF1R antagonist.

## 11.9 Anti-glucocorticoid Therapy for Mood Disorders

Patients with psychotic depression exhibit the highest rates of DEX non-suppression compared to non-psychotic major depression patients and normal volunteers.<sup>64,65</sup> In psychotic major depression, high cortisol concentrations are associated with hyperactivity of dopamine neurons, and it is this effect that has



been hypothesized to contribute to the psychosis pathognomonic of psychotic depression.<sup>66</sup> One novel treatment option for psychotic depression that has been suggested is the reduction of high cortisol levels using agents that block the synthesis of cortisol, such as metyrapone, aminoglutethamide and the antifungal agent ketoconazole. However, all of these drugs are problematic because of side-effects and potential untoward drug–drug interactions. Therefore, if cortisol synthesis cannot be reduced, then another approach would be to antagonize the effects of cortisol.

Corticosteroids bind to two forms of intra-cellular steroid receptors, the “type 1” mineralocorticoid (MR) receptor (MR) with high affinity ( $K_d = 0.5$  nM) and the “type 2” glucocorticoid receptor (GR) with a lower affinity ( $K_d = 5$  nM). Cortisol is similar in structure to the sex steroid progesterone and also the potent progesterone antagonist, mifepristone, **8** (RU486), which is FDA approved for reproductive indications. At high doses mifepristone antagonizes GRs, but not MRs, and there is considerable evidence that it has efficacy in the management of psychotic depression.<sup>67–72</sup> One hypothesized mechanism for this is that antagonism of GRs causes MR expression to be up-regulated, thus providing a greater capacity for HPA axis feedback regulation.<sup>73</sup> A number of clinical trials support the use of mifepristone in the treatment of depression. In one early case report, Nieman *et al.* reported the use of a 9-week escalating dose of mifepristone to successfully treat a case of Cushing’s syndrome (ACTH overproduction).<sup>74</sup> It was also noted in that 1985 case report that the patient’s suicidal tendencies also resolved. Based upon this work, Murphy *et al.* hypothesized in 1993 that longer-term treatments with high-dose mifepristone might be useful to treat major depression.<sup>67</sup> They conducted a trial in four patients who were refractory to other treatments. The patients received 200 mg/day mifepristone over 8 weeks. There was a decrease in Hamilton Rating Scale for Depression (HAMD) scores from patients in the study, though the number of patients studied was too small for the results to be conclusive.

In 2001, Belanoff *et al.* reported the effects of 600 mg/day mifepristone over the course of 4 days in 5 patients with psychotic major depression (PMD) and found significant decreases in both HAMD and Brief Psychiatric Rating Scale (BPRS) scores compared to placebo.<sup>75</sup> The same group built upon this study by testing 50, 600 or 1,200 mg/day of mifepristone over the course of 7 days in 30 patients with PMD. Again, improvements in both HAMD and BPRS scores were observed.<sup>76</sup> Subsequent larger clinical trials in PMD and bipolar disorder have confirmed the earlier reports of the efficacy of mifepristone.<sup>69,77,78</sup> Three of the largest studies, all sponsored by Corcept, with 221, 258 and 443 patient cohorts were treated with placebo or mifepristone (doses ranged from 300–1,200 mg/day) over 7 days.<sup>68,72,79</sup> In all three phase II studies, there were positive effects seen with mifepristone treatment, including a correlation between mifepristone plasma concentrations and clinical improvement, which even persisted for several weeks after mifepristone discontinuation.<sup>79</sup> Corcept is currently recruiting into additional phase III trials for mifepristone. It is encouraging that virtually all the mifepristone trials reported some promising

results. Short-term mifepristone treatment may well prove to be useful in the management of psychotic depression.

Because mifepristone has significant and potentially deleterious side-effects associated with its affinity for the progesterone receptor, the development of more selective GR antagonists is among the most attractive novel approaches to treat psychotic mood disorders. There have been some reports of such compounds, particularly RU-43044, **9**, and CORT-108297, **10** (Figure 11.4). RU-43044 is a selective GR antagonist that shows some antidepressant-like effects in mice, though chronic administration inexplicably leads to more depressive like outcomes in mice.<sup>80</sup> CORT-108297 has shown some anti-obesity results in mouse models of obesity,<sup>81</sup> but little work has been done in psychiatric disorders. However, the ultimate clinical utility of selective GR antagonists remains unclear.<sup>82–84</sup>

## 11.10 Glucocorticoid and Mineralocorticoid Receptor Activation in the Treatment of Non-psychotic and Anxious Mood Disorders

HPA axis feedback both in the brain and in the pituitary is achieved *via* cortisol activation of glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). As noted above, the MRs have a higher sensitivity to cortisol than the GRs, and therefore feedback primarily occurs first at MRs in the hippocampus.<sup>85</sup> Under very stressful situations, and increased cortisol levels, the GRs are activated and additional feedback inhibition of the HPA axis occurs in a number of brain regions and the anterior pituitary.<sup>85</sup> Because non-psychotic patients tend to have an overdrive in HPA axis output and cortisol levels, the MR feedback regulation system is likely already saturated and thus makes a poor candidate for further activation to reduce HPA activity. The GR system, however, makes a much more attractive target, not only due to the lower rate of activation but also due to its broader distribution profile. Mood disorder patients also display poor feedback regulation of the HPA *via* GRs.<sup>86,87</sup> Clinical studies have revealed polymorphisms of GRs and related molecules and mood disorders (discussed below).<sup>88</sup>

There are several GR- and MR-specific agonists that have been developed, and the hypothesis behind their use for mood disorders is that by using an agonist to potentiate the feedback inhibition of CRF and ACTH release, HPA axis tone can be reduced. Endogenous corticoids may be excellent therapeutic candidates because of their long plasma half-life and brain penetration. Perhaps better than the endogenous glucocorticoids are their synthetic analogues, such as dexamethasone, **11**, and prednisone, **12** (Figure 11.4). Dexamethasone is a potent synthetic GR agonist that is 25 times more potent than cortisol. DEX was developed in 1957 by Merck.<sup>89</sup> It has a very long half-life *in vivo* and is currently being used clinically to treat inflammation and to offset side-effects of chemotherapy, among other uses. There have been a few clinical trials using DEX to treat non-psychotic depression,<sup>90,91</sup> and PTSD (as of this writing,

PTSD/DEX trials are in recruiting phase). In the first reported examination of DEX in depressed patients, McClure and Cleghorn administered 0.75 mg of DEX in combination with imipramine and noted a positive effect.<sup>90</sup> In later studies by Arana and colleagues, a number of trials were conducted. In one with 37 patients treated for 14 days, DEX treatment was superior to placebo in reducing depressive symptoms.<sup>91</sup>

Some of the most convincing data support the potential use of DEX in anxiety disorders and particularly PTSD. PTSD patients tend to have lower cortisol levels and are more sensitive than normal volunteers to DEX suppression, even though CSF CRF levels are elevated.<sup>42,92</sup> PTSD patients may have reduced capacity for GR-mediated feedback inhibition of HPA tone due to such low cortisol levels (though the reason for low cortisol is not well understood), and that activation of the GR by DEX, for example, could be therapeutically useful. In two studies of DEX treatment in PTSD patients, one of combat veterans and the other of sexually abused adolescents, reductions in ACTH levels were reported after DEX administration, though neither study attempted to evaluate the behavioural effects of DEX.<sup>93,94</sup> A more recent study examined the effects of DEX on fear-potentiated startle (FPS) in 33 PTSD patients and 67 controls.<sup>95</sup> Half of the subjects were treated with a single dose of DEX, while the others were untreated. DEX reduced fear-potentiated startle and there was a correlation with cortisol levels and subject performance in FPS response suggesting that DEX may reduce exaggerated fear in PTSD patients. As noted above, additional clinical trials are currently underway in PTSD patients to determine whether DEX can be useful to suppress HPA axis dysregulation. It remains unclear whether the potentially beneficial effects of GR or MR agonists are due to regulations of HPA axis activity or due to extra-endocrine CNS effects.

Unfortunately there are well-characterized side-effects associated with administration of DEX or prednisone. Depression, mania, psychosis and delirium have all been reported after even a single acute dose of prednisone,<sup>96</sup> though these are largely isolated case reports. More informative perhaps are the behavioural and cognitive effects of high-dose corticosteroid administration, which are associated with a high incidence of depression, anxiety and hypomania.<sup>97</sup> A similar study in asthma patients with sporadic dosing also revealed mood disturbances.<sup>98</sup> A number of other effects on cognitive function, learning and memory, brain atrophy and corticosteroid withdrawal have all been described and are reviewed elsewhere.<sup>99</sup> However, with the successful use of glucocorticoids for a variety of medical disorders over the span of decades, these side-effects may not pose an insurmountable barrier to their use in psychiatric patients.

## **11.11 Neuropeptides and HPA Axis Control of Mood Disorders**

A variety of neuropeptide signalling systems have been shown to be involved in mood dysregulation. While these systems may not be considered primary

components of the HPA axis, there is some indication that they are important in the pathophysiology of mood and anxiety disorders and may dysregulate HPA axis activity. Substance P, galanin and, not least, neuropeptide Y receptors are all considered viable therapeutic targets for treating mood and anxiety disorders.<sup>62,100</sup> The roles of these neuropeptides and their potential use in treating mood disorders have been reviewed extensively elsewhere. Here we will briefly describe the relative contributions, if known, of the neuropeptides to HPA axis dysfunction as well as the current state of therapeutic development for these novel targets.

### 11.11.1 Substance P

Substance P (SP) is an undecapeptide belonging to the tachykinin family of peptides. SP and its receptor neurokinin-1 (NK1) are most notably linked to pain signalling in the brain. SP is also involved in relaying stress, anxiety, nausea and inflammatory endocrine signals and has been considered an attractive target for development of a novel anxiolytic and possibly anti-depressant.<sup>100–104</sup> SP is colocalized with CRF in the hypothalamus and it has been suggested that SP controls the release of CRF.<sup>105,106</sup> Much of the evidence supporting a role of SP in mood regulation comes from evaluating the effects of exogenous SP administration or NK1 genetic deletions that collectively show a variety of anxious and depressive-like phenotypes.<sup>107</sup> The clinical utility of NK1 antagonists has been assessed in clinical trials. In one such trial, the NK1 receptor antagonist, aprepitant, **13** (Emend, MK-869) (Figure 11.5), was compared to an SSRI, paroxetine, and placebo.<sup>108</sup> Aprepitant performed just as well as the SSRI in reducing depression, as measured by Hamilton Rating Scale for Depression (HAMD) scores, but with significantly lower rates of some side-effects such as sexual dysfunction.<sup>108</sup> A similar study, with an analogue of aprepitant, L759274, by the same group had similar findings.<sup>109</sup> However, these two phase II trials were overshadowed by a larger phase III study with 2,500 patients treated with placebo, paroxetine or aprepitant, in which there was no evidence of efficacy of aprepitant compared to placebo for major depressive

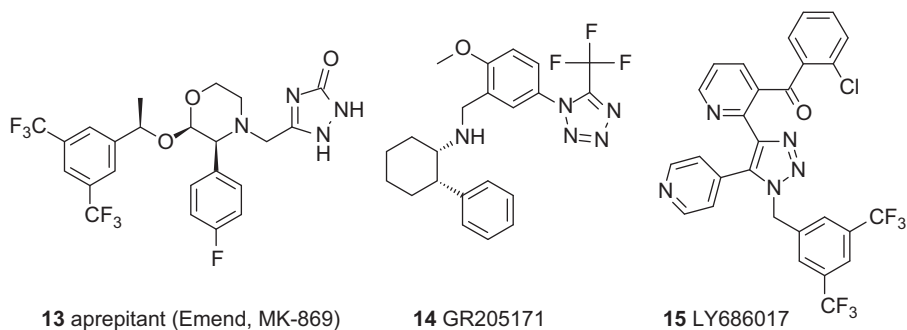


Figure 11.5

disorder.<sup>110</sup> Paroxetine, in contrast, was superior in efficacy to both placebo and aprepitant.

Additional clinical trials have yielded similarly mixed results. Furmark *et al.* treated 36 patients for 4 weeks with GR205171, **14** (Figure 11.5), a selective NK1 antagonist, and then measured cerebral blood flow during a stressful public speaking task. Patients responded much better to social stress in the drug treated group compared to placebo.<sup>111</sup> In 2010 investigators at Eli Lilly published a study with 189 social anxiety disorder (SAD) patients and found no effect of up to 50 mg/day of the NK1 antagonist LY686017, **15**, and placebo in the Liebowitz Social Anxiety Scale (LSAS).<sup>112</sup> Recently, Matthew and colleagues published a study in which PTSD patients (defined by Clinician-Administered PTSD Scale (CAPS) scores of greater than 50) were treated for 8 weeks with GR205171.<sup>113</sup> No significant effect of the NK1 antagonist was observed on average CAPS score or the number of patients with reduced CAPS score compared to placebo. Although there remain a number of NK1 antagonists in development,<sup>114</sup> and aprepitant is currently FDA approved to treat nausea in chemotherapy patients, the potential utility in treating mood or anxiety disorders with NK1 antagonists is tenuous. Indeed, Fujimura *et al.* published a Positron Emission Tomographic (PET) study with [<sup>18</sup>F]SPA-RQ, a selective NK1 receptor antagonist.<sup>115</sup> Patients (n = 14) with panic disorder exhibited lower NK1 receptor binding density compared to healthy controls. In light of these results, an agonist approach might be more useful. Alternatively, NK1 antagonists have not yet been tested in the subset of depressive or anxious patients with chronic inflammation where substance P levels may also be chronically high, a subset in which they may be more efficacious.<sup>116</sup>

### 11.11.2 Neuropeptide Y

Neuropeptide Y (NPY) is a 36 amino acid peptide that is found throughout the body and has a variety of physiological roles, some of which have been linked to the HPA axis, mostly by way of control of CRF release.<sup>117</sup> NPY has been postulated to be involved in a number of psychiatric diseases including alcoholism and depression.<sup>118,119</sup> Unfortunately, there are no clinically useful NPY receptor agonists or antagonists, though development work is underway.<sup>119</sup> Therefore, the suitability of NPY receptor ligands to treat mood disorders is based entirely on animal experiments and clinical observations, also reviewed elsewhere.<sup>60,120,121</sup>

NPY is released in stressful situations both in animals and humans, and low CSF levels of NPY are associated with increasing depression severity and correlate inversely with anxiety symptoms in depressed patients.<sup>122</sup> Therefore an increase in NPY levels has been hypothesized to alleviate mood disorders. This increase in NPY signalling is thought to require increased Y1 receptor signal transmission. This increase in Y1 receptor signalling can be accomplished in two ways: first Y1R agonists can be used or antagonism of pre-synaptic Y2 auto-receptor feedback inhibition can lead to increased NPY

levels, a strategy that our group and others are pursuing.<sup>119,123</sup> Lacking optimized molecules, it is unclear whether an NPY-directed strategy to treat mood disorders will be clinically useful.

### 11.11.3 Galanin

Galanin, a 30 amino acid peptide with significant species variation, is involved in a number of physiologic processes including metabolism, reproduction and mood. Galanin 1 receptor knockout mice exhibit increased anxiety and central injection of galanin has been shown to exert differential effects on cognitive function.<sup>124</sup> The contribution of galanin to normal or pathological mood states is unclear.<sup>125</sup> An agonist approach to activate the galanin 1, 2 and/or 3 receptors might prove useful. To this end, there are two widely used agonists of the G1R and G2R, galnon and galmic, both non-peptide small-molecule G1R and G2R non-selective agonists.<sup>125–128</sup> Galnon has been shown to possess anxiolytic properties, though there may be additional effects of galnon not mediated by the galanin receptors.<sup>129</sup> Much less is known about galmic.<sup>128,130</sup> Galanin 3 receptor antagonists have been proposed to be useful in treating anxiety disorders based largely on animal experiments.<sup>131</sup> HT-2157 is a selective galanin 3 receptor antagonist that is scheduled to be studied in phase II trials.

## 11.12 FKBP5 as a Genetically Linked HPA Axis Therapeutic Target

A number of HPA axis genes have been identified that have been shown to be genetic risk factors for mood disorders. The most notable is that which codes for the CRF1 receptor (*crfr1*).<sup>6,57,132</sup> There have been numerous other studies linking one locus or another to mood disorders. We will not discuss all of these studies here due to the numerous outstanding reviews available.<sup>6,57,88,133–135</sup> Rather, we will focus on FKBP5 (aka FKBP51, FK506 binding protein 51, hsp58), which has been genetically linked to anxiety, depression and PTSD.<sup>136,137</sup>

In the context of the HPA axis, FKBP5 works with hsp90 to regulate glucocorticoid receptor sensitivity.<sup>137</sup> Briefly, hsp90 (along with numerous other proteins) acts in the cell to help proteins properly fold and evade degradation. Hsp90 action is supported by co-chaperones in the cell as well, one of these being the immunophilin FKBP5.<sup>137</sup> Immunophilins such as FKBP5 and cyclophilin convert proline imidine bonds from the *cis* to the *trans* configuration, and often interact with chaperones such as hsp90 in the cell.<sup>137,138</sup> In GR-containing cells, hsp90 and FKBP5 indeed both bind to the GR as chaperones during protein folding and maturation.<sup>139</sup> When the GR is bound with hsp90 and FKBP5, it becomes less sensitive to the presence of cortisol.<sup>139</sup> When FKBP5 is over-expressed either *in vitro* or *in vivo*, there is a significant reduction in cortisol action at GRs.<sup>137,139,140</sup> Indeed, FKBP5 is up-regulated by GR activation, suggesting that FKBP5 is an intra-cellular negative feedback protein.<sup>141</sup> Therefore, it is not surprising that FKBP5 polymorphisms have been shown to be associated with a variety of mood disorders.<sup>136,137</sup>



FKBP5 was originally identified based upon its ability to bind FK506 (aka fujimycin, tacrolimus), a common immunosuppressant derived from *Streptomyces tsukubaensis*, and FDA approved to suppress the immune system for organ transplant patients.<sup>142</sup> FK506 is an inhibitor of FKBP5, but also of the other FKBP family members, FKBP12 and FKBP4, making this a poor candidate for clinical trials in mood disorders. To our knowledge there are no other efforts to develop additional, selective FKBP5 antagonists. However, the development of such antagonists might be useful to reduce the FKBP5 desensitizing effects on GRs and allow feedback inhibition of HPA axis tone. Work in this area is of great interest, though a great deal of validation, especially at potential off-target effects, is necessary.

## 11.13 Conclusions and Expert Opinion

We have outlined a number of different components of the HPA axis that represent attractive targets to treat mood disorders. The first and strongest therapeutic candidates, and most directly linked to the HPA axis, are glucocorticoid receptor antagonists for depression and agonists for PTSD. Interestingly, there are two such ligands already FDA approved for other indications: mifepristone (antagonist) and dexamethasone (agonist) and clinical trials with these compounds for psychotic depression and PTSD, respectively, are ongoing. The CRF1 receptor is in need of a clinical trial in an enriched sample of depressed patients with a history of childhood abuse or neglect who exhibit chronically elevated CRF, ACTH and cortisol levels. Indeed, in any mood disorder, the patient population unequivocally shown to hypersecrete CRF would be of particular interest with regard to CRF1 receptor antagonists, and in line with the burgeoning field of personalized medicine. CRF2 receptor antagonists certainly deserve further scrutiny as well. The class of neuropeptide modulator systems that lie just outside and somewhat upstream of the HPA axis, namely NPY and galanin, represents a highly interesting strategy to treat mood disorders but awaits molecule optimization. Finally, targets that have been found by genetic and genomic screens, such as FKBP5, are potentially interesting but this field is still very much in its infancy.

## Financial Disclosures

*Declaration of Financial/Propriety Interest for Shaun P. Brothers, Ph.D. (December 2009 – Present)*

Research/Grants: None

Speakers Bureau: None

Consulting: None

Stockholder: Repligen Corp., Cortex Pharm. Inc.

Other Financial Interests: None

Patents: None

Scientific Advisory Boards: None



Board of Directors: None

Income Sources and Equity of \$10,000 or more: None

*Declaration of Financial/Propriety Interest for Claes Wahlestedt, M.D., Ph.D. (December 2009 – Present)*

Research/Grants: National Institutes of Health (NIH)

Speakers Bureau: None

Consulting: OPKO CURNA, LLC

Stockholder: CURNA Inc.

Other Financial Interests: None

Patents: None pertaining to this manuscript

Scientific Advisory Boards: OPKO CURNA, LLC; Florida Center for Brain Tumor Research; Biomedical Research Advisory Council of Florida

Board of Directors: St Mary's Medical Center, Miami Winter Symposia; Florida Blood Centers; Florida Center for Universal Research to Eradicate Disease (FLCURED)

Income Sources and Equity of \$10,000 or more: OPKO CURNA, LLC

*Declaration of Financial/Propriety Interest for Charles B. Nemeroff, M.D., Ph.D. (December 2009 – Present)*

Research/Grants: National Institutes of Health (NIH), Agency for Healthcare Research and Quality (AHRQ)

Speakers Bureau: None

Consulting: Xhale, Takeda, SK Pharma

Stockholder: CeNeRx BioPharma, NovaDel Pharma, Inc., PharmaNeuroBoost, Revaax Pharma, Xhale

Other Financial Interests: CeNeRx BioPharma, PharmaNeuroBoost

Patents: Method and devices for transdermal delivery of lithium (US 6,375,990B1)

Method of assessing antidepressant drug therapy *via* transport inhibition of monoamine neurotransmitters by *ex vivo* assay (US 7,148,027B2)

Scientific Advisory Boards: American Foundation for Suicide Prevention (AFSP), CeNeRx BioPharma, National Alliance for Research on Schizophrenia and Depression (NARSAD), NovaDel Pharma, Inc., PharmaNeuroBoost, Anxiety Disorders Association of America (ADAA)

Board of Directors: AFSP, NovaDel Pharma, Inc., Mt. Cook Pharma (2010)

Income Sources and Equity of \$10,000 or more: PharmaNeuroBoost, CeNeRx, NovaDel Pharma, American Psychiatric Publishing, Xhale

## Grant Support

This work was supported in part by the following NIH grants: 5R01DA025983, 5R01MH084880, 7R01NS063974.

## References

1. W. Vale, J. Spiess, C. Rivier and J. Rivier, *Science*, 1981, **213**, 1394–1397.
2. L. Arborelius, M. J. Owens, P. M. Plotsky and C. B. Nemeroff, *J. Endocrinol.*, 1999, **160**, 1–12.
3. X. F. Li, A. M. Knox and K. T. O'Byrne, *Brain Res.*, 2010, **1364**, 153–163.
4. E. P. Zorrilla, Y. Tache and G. F. Koob, *Trends Pharmacol. Sci.*, 2003, **24**, 421–427.
5. E. G. Lowery and T. E. Thiele, *CNS Neurol. Disord. Drug Targets*, 2010, **9**, 77–86.
6. E. B. Binder and C. B. Nemeroff, *Mol. Psychiatry*, 2010, **15**, 574–588.
7. D. H. Henneman, M. D. Altschule and R. M. Goncz, *AMA Arch. Internal Med.*, 1955, **95**, 241–246.
8. D. J. McClure, *J. Psychosom. Res.*, 1966, **10**, 189–195.
9. C. F. Gillespie and C. B. Nemeroff, *Psychosom. Med.*, 2005, **67**(1), S26–S28.
10. D. G. Baker, S. A. West, W. E. Nicholson, N. N. Ekhtor, J. W. Kasckow, K. K. Hill, A. B. Bruce, D. N. Orth and T. D. Geraciotti, Jr, *Am. J. Psychiatry*, 1999, **156**, 585–588.
11. J. D. Bremner, J. Licinio, A. Darnell, J. H. Krystal, M. J. Owens, S. M. Southwick, C. B. Nemeroff and D. S. Charney, *Am. J. Psychiatry*, 1997, **154**, 624–629.
12. C. M. Banki, G. Bissette, M. Arato, L. O'Connor and C. B. Nemeroff, *Am. J. Psychiatry*, 1987, **144**, 873–877.
13. D. A. Axelson, P. M. Doraiswamy, W. M. McDonald, O. B. Boyko, L. A. Tupler, L. J. Patterson, C. B. Nemeroff, E. H. Ellinwood, Jr and K. R. Krishnan, *Psychiatry Res.*, 1993, **47**, 163–173.
14. C. B. Nemeroff, E. Widerlov, G. Bissette, H. Walleus, I. Karlsson, K. Eklund, C. D. Kilts, P. T. Loosen and W. Vale, *Science*, 1984, **226**, 1342–1344.
15. L. L. Carpenter, A. R. Tyrka, C. J. McDougale, R. T. Malison, M. J. Owens, C. B. Nemeroff and L. H. Price, *Neuropsychopharmacology*, 2004, **29**, 777–784.
16. M. D. Fossey, R. B. Lydiard, J. C. Ballenger, M. T. Laraia, G. Bissette and C. B. Nemeroff, *Biol. Psychiatry*, 1996, **39**, 703–707.
17. C. B. Nemeroff, M. J. Owens, G. Bissette, A. C. Andorn and M. Stanley, *Arch. Gen. Psychiatry*, 1988, **45**, 577–579.
18. Z. Merali, L. Du, P. Hrdina, M. Palkovits, G. Faludi, M. O. Poulter and H. Anisman, *J. Neurosci.*, 2004, **24**, 1478–1485.
19. E. I. Flandreau, K. J. Ressler, M. J. Owens and C. B. Nemeroff, *Psychoneuroendocrinology*, 2012, **37**(1), 27.
20. C. Stetler and G. E. Miller, *Psychosom. Med.*, 2011, **73**, 114–126.
21. C. Heim, D. J. Newport, R. Bonsall, A. H. Miller and C. B. Nemeroff, *Am. J. Psychiatry*, 2001, **158**, 575–581.
22. C. B. Nemeroff, K. R. Krishnan, D. Reed, R. Leder, C. Beam and N. R. Dunnick, *Arch. Gen. Psychiatry*, 1992, **49**, 384–387.

23. C. Heim and C. B. Nemeroff, *Biol. Psychiatry*, 1999, **46**, 1509–1522.
24. E. Holsboer-Trachsler, R. Stohler and M. Hatzinger, *Psychiatry Res.*, 1991, **38**, 163–171.
25. F. Holsboer, *J. Affect. Disord.*, 2001, **62**, 77–91.
26. A. W. Zobel, A. Yassouridis, R. M. Frieboes and F. Holsboer, *Am. J. Psychiatry*, 1999, **156**, 949–951.
27. G. N. Neigh, K. Karelina, N. Zhang, E. R. Glasper, M. J. Owens, P. M. Plotsky, C. B. Nemeroff and A. C. Devries, *J. Cereb. Blood Flow Metab.*, 2009, **29**, 1673–1682.
28. M. A. Whooley, *JAMA*, 2006, **295**, 2874–2881.
29. J. B. Regard, I. T. Sato and S. R. Coughlin, *Cell*, 2008, **135**, 561–571.
30. P. Timpl, R. Spanagel, I. Sillaber, A. Kresse, J. M. Reul, G. K. Stalla, V. Blanquet, T. Steckler, F. Holsboer and W. Wurst, *Nat. Genet.*, 1998, **19**, 162–166.
31. J. H. Kehne and C. K. Cain, *Pharmacol. Ther.*, 2010, **128**, 460–487.
32. D. A. Gutman, M. J. Owens, K. V. Thiruvikraman and C. B. Nemeroff, *Neuropharmacology*, 2011, **60**, 1135–1141.
33. G. F. Koob, *Brain Res.*, 2009, **1293**, 61–75.
34. M. Eaves, K. Thatcher-Britton, J. Rivier, W. Vale and G. F. Koob, *Peptides*, 1985, **6**, 923–926.
35. A. W. Zobel, T. Nickel, H. E. Kunzel, N. Ackl, A. Sonntag, M. Ising and F. Holsboer, *J. Psychiatr. Res.*, 2000, **34**, 171–181.
36. M. Ising, U. S. Zimmermann, H. E. Kunzel, M. Uhr, A. C. Foster, S. M. Learned-Coughlin, F. Holsboer and D. E. Grigoriadis, *Neuropsychopharmacology*, 2007, **32**, 1941–1949.
37. B. Binneman, D. Feltner, S. Kolluri, Y. Shi, R. Qiu and T. Stiger, *Am. J. Psychiatry*, 2008, **165**, 617–620.
38. V. Coric, H. H. Feldman, D. A. Oren, A. Shekhar, J. Pultz, R. C. Dockens, X. Wu, K. A. Gentile, S. P. Huang, E. Emison, T. Delmonte, B. B. D'souza, D. L. Zimbroff, J. A. Grebb, A. W. Goddard and E. G. Stock, *Depress. Anxiety*, 2010, **27**, 417–425.
39. C. S. Hubbard, J. S. Labus, J. Bueller, J. Stains, B. Suyenobu, G. E. Dukes, D. L. Kelleher, K. Tillisch, B. D. Naliboff and E. A. Mayer, *J. Neurosci.*, 2011, **31**, 12491–12500.
40. C. Heim, D. J. Newport, S. Heit, Y. P. Graham, M. Wilcox, R. Bonsall, A. H. Miller and C. B. Nemeroff, *JAMA*, 2000, **284**, 592–597.
41. C. Heim, T. Mletzko, D. Purselle, D. L. Musselman and C. B. Nemeroff, *Biol. Psychiatry*, 2008, **63**, 398–405.
42. C. B. Nemeroff, J. D. Bremner, E. B. Foa, H. S. Mayberg, C. S. North and M. B. Stein, *J. Psychiatr. Res.*, 2006, **40**, 1–21.
43. A. B. Amstadter, N. R. Nugent, B. Z. Yang, A. Miller, R. Siburian, P. Moorjani, S. Haddad, A. Basu, J. Fagerness, G. Saxe, J. W. Smoller and K. C. Koenen, *Dis. Markers*, 2011, **30**, 89–99.
44. G. Polanczyk, A. Caspi, B. Williams, T. S. Price, A. Danese, K. Sugden, R. Uher, R. Poulton and T. E. Moffitt, *Arch. Gen. Psychiatry*, 2009, **66**, 978–985.

45. A. C. Chen, N. Manz, Y. Tang, M. Rangaswamy, L. Almasy, S. Kuperman, J. Nurnberger, Jr, S. J. O'Connor, H. J. Edenberg, M. A. Schuckit, J. Tischfield, T. Foroud, L. J. Bierut, J. Rohrbach, J. P. Rice, A. Goate, V. Hesselbrock and B. Porjesz, *Alcohol. Clin. Exp. Res.*, 2010, **34**, 988–996.
46. R. Ciccocioppo, D. R. Gehlert, A. Ryabinin, S. Kaur, A. Cippitelli, A. Thorsell, A. D. Le, P. A. Hipkind, C. Hamdouchi, J. Lu, E. J. Hembre, J. Cramer, M. Song, D. McKinzie, M. Morin, D. Economidou, S. Stopponi, N. Cannella, S. Braconi, M. Kallupi, G. de Guglielmo, M. Massi, D. T. George, J. Gilman, J. Hersh, J. T. Tauscher, S. P. Hunt, D. Hommer and M. Heilig, *Alcohol*, 2009, **43**, 491–498.
47. M. Heilig and M. Egli, *Pharmacol. Ther.*, 2006, **111**, 855–876.
48. S. C. Coste, R. A. Kesterson, K. A. Heldwein, S. L. Stevens, A. D. Heard, J. H. Hollis, S. E. Murray, J. K. Hill, G. A. Pantely, A. R. Hohimer, D. C. Hatton, T. J. Phillips, D. A. Finn, M. J. Low, M. B. Rittenberg, P. Stenzel and M. P. Stenzel-Poore, *Nat. Genet.*, 2000, **24**, 403–409.
49. T. L. Bale, R. Picetti, A. Contarino, G. F. Koob, W. W. Vale and K. F. Lee, *J. Neurosci.*, 2002, **22**, 193–199.
50. T. Kishimoto, J. Radulovic, M. Radulovic, C. R. Lin, C. Schrick, F. Hooshmand, O. Hermanson, M. G. Rosenfeld and J. Spiess, *Nat. Genet.*, 2000, **24**, 415–419.
51. R. Gerlai, *Trends Neurosci.*, 1996, **19**, 177–181.
52. R. L. Hauger, V. Risbrough, O. Brauns and F. M. Dautzenberg, *CNS Neurol. Disord. Drug Targets*, 2006, **5**, 453–479.
53. D. N. Orth and C. D. Mount, *Biochem. Biophys. Res. Commun.*, 1987, **143**, 411–417.
54. E. Potter, D. P. Behan, W. H. Fischer, E. A. Linton, P. J. Lowry and W. W. Vale, *Nature*, 1991, **349**, 423–426.
55. R. J. Herringa, P. H. Roseboom and N. H. Kalin, *Neuropsychopharmacology*, 2006, **31**, 1822–1831.
56. A. J. Rush, M. Fava, S. R. Wisniewski, P. W. Lavori, M. H. Trivedi, H. A. Sackeim, M. E. Thase, A. A. Nierenberg, F. M. Quitkin, T. M. Kashner, D. J. Kupfer, J. F. Rosenbaum, J. Alpert, J. W. Stewart, P. J. McGrath, M. M. Biggs, K. Shores-Wilson, B. D. Lebowitz, L. Ritz and G. Niederehe, *Control. Clin. Trials*, 2004, **25**, 119–142.
57. E. B. Binder, M. J. Owens, W. Liu, T. C. Deveau, A. J. Rush, M. H. Trivedi, M. Fava, B. Bradley, K. J. Ressler and C. B. Nemeroff, *Arch. Gen. Psychiatry*, 2010, **67**, 369–379.
58. R. J. Herringa, S. A. Nanda, D. T. Hsu, P. H. Roseboom and N. H. Kalin, *Brain Res. Mol. Brain Res.*, 2004, **131**, 17–25.
59. M. A. Faghihi and C. Wahlestedt, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 637–643.
60. S. Rotzinger, D. A. Lovejoy and L. A. Tan, *Peptides*, 2010, **31**, 736–756.
61. C. Murgatroyd, A. V. Patchev, Y. Wu, V. Micale, Y. Bockmuhl, D. Fischer, F. Holsboer, C. T. Wotjak, O. F. Almeida and D. Spengler, *Nat. Neurosci.*, 2009, **12**, 1559–1566.

62. A. Holmes, M. Heilig, N. M. Rupniak, T. Steckler and G. Griebel, *Trends Pharmacol. Sci.*, 2003, **24**, 580–588.
63. C. Serradeil-Le Gal, J. Wagnon, 3rd, B. Tonnerre, R. Roux, G. Garcia, G. Griebel and A. Aulombard, *CNS Drug Rev.*, 2005, **11**, 53–68.
64. D. L. Evans, G. B. Burnett and C. B. Nemeroff, *Am. J. Psychiatry*, 1983, **140**, 586–589.
65. J. C. Nelson and J. M. Davis, *Am. J. Psychiatry*, 1997, **154**, 1497–1503.
66. A. F. Schatzberg, A. J. Rothschild, P. J. Langlais, E. D. Bird and J. O. Cole, *J. Psychiatr. Res.*, 1985, **19**, 57–64.
67. B. E. Murphy, D. Filipini and A. M. Ghadirian, *J. Psychiatry Neurosci. JPN*, 1993, **18**, 209–213.
68. C. DeBattista, J. Belanoff, S. Glass, A. Khan, R. L. Horne, C. Blasey, L. L. Carpenter and G. Alva, *Biol. Psychiatry*, 2006, **60**, 1343–1349.
69. A. H. Young, P. Gallagher, S. Watson, D. Del-Estal, B. M. Owen and I. N. Ferrier, *Neuropsychopharmacology*, 2004, **29**, 1538–1545.
70. C. B. Nemeroff, *Hum. Psychopharmacol.*, 2002, **17**(1), S13–S16.
71. K. R. Krishnan, D. Reed, W. H. Wilson, W. B. Saunders, J. C. Ritchie, C. B. Nemeroff and B. J. Carroll, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 1992, **16**, 913–920.
72. C. M. Blasey, T. S. Block, J. K. Belanoff and R. L. Roe, *J. Clin. Psychopharmacol.*, 2011, **31**, 436–440.
73. C. G. Bachmann, A. C. Linthorst, F. Holsboer and J. M. Reul, *Neuropsychopharmacology*, 2003, **28**, 1056–1067.
74. L. K. Nieman, G. P. Chrousos, C. Kellner, I. M. Spitz, B. C. Nisula, G. B. Cutler, G. R. Merriam, C. W. Bardin and D. L. Loriaux, *J. Clin. Endocrinol. Metab.*, 1985, **61**, 536–540.
75. J. K. Belanoff, B. H. Flores, M. Kalezhan, B. Sund and A. F. Schatzberg, *J. Clin. Psychopharmacol.*, 2001, **21**, 516–521.
76. J. K. Belanoff, A. J. Rothschild, F. Cassidy, C. DeBattista, E. E. Baulieu, C. Schold and A. F. Schatzberg, *Biol. Psychiatry*, 2002, **52**, 386–392.
77. G. M. Simpson, A. El Sheshai, N. Loza, S. J. Kingsbury, M. Fayek, A. Rady and W. Fawzy, *J. Clin. Psychiatry*, 2005, **66**, 598–602.
78. B. H. Flores, H. Kenna, J. Keller, H. B. Solvason and A. F. Schatzberg, *Neuropsychopharmacology*, 2006, **31**, 628–636.
79. C. M. Blasey, C. DeBattista, R. Roe, T. Block and J. K. Belanoff, *Contemp. Clin. Trials*, 2009, **30**, 284–288.
80. Y. Ago, S. Arikawa, M. Yata, K. Yano, M. Abe, K. Takuma and T. Matsuda, *Neuropharmacology*, 2008, **55**, 1355–1363.
81. T. Asagami, J. K. Belanoff, J. Azuma, C. M. Blasey, R. D. Clark and P. S. Tsao, *J. Nutr. Metab.*, 2011, **2011**, 235389.
82. Q. Y. Li, M. Zhang, T. M. Hallis, T. A. Derosier, J. M. Yue, Y. Ye, D. E. Mais and M. W. Wang, *Biochem. Biophys. Res. Commun.*, 2010, **391**, 1531–1536.
83. A. R. Brown, M. Bosies, H. Cameron, J. Clark, A. Cowley, M. Craighead, M. A. Elmore, A. Firth, R. Goodwin, S. Goutcher, E. Grant, M. Grassie, S. J. Grove, N. M. Hamilton, H. Hampson, A. Hillier, K. K.

- Ho, M. Kiczun, C. Kingsbury, S. G. Kultgen, P. T. Littlewood, S. J. Lusher, S. Macdonald, L. McIntosh, T. McIntyre, A. Mistry, J. R. Morphy, O. Nimz, M. Ohlmeyer, J. Pick, Z. Rankovic, B. Sherborne, A. Smith, M. Speake, G. Spinks, F. Thomson, L. Watson and M. Weston, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 137–140.
84. J. Rimland, A. Dunne, S. S. Hunjan, R. Sasse, I. Uings, D. Montanari, M. Caivano, P. Shah, D. Standing, D. Gray, D. Brown, W. Cairns, R. Trump, P. W. Smith, N. Bertheleme, P. D'Alessandro, S. Gul, M. Vimal, D. N. Smith and S. P. Watson, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2340–2343.
85. J. G. Tasker and J. P. Herman, *Stress*, 2011, **14**, 398–406.
86. G. N. Neigh and C. B. Nemeroff, *Trends Endocrinol. Metab.*, 2006, **17**, 124–125.
87. S. Ridder, S. Chourbaji, R. Hellweg, A. Urani, C. Zacher, W. Schmid, M. Zink, H. Hortnagl, H. Flor, F. A. Henn, G. Schutz and P. Gass, *J. Neurosci.*, 2005, **25**, 6243–6250.
88. S. Claes, *Ann. NY Acad. Sci.*, 2009, **1179**, 216–228.
89. E. W. Boland, *Ann. Rheum. Dis.*, 1958, **17**, 376–382.
90. D. J. McClure and R. A. Cleghorn, *Can. Psychiatr. Assoc. J.*, 1968, **13**, 477–488.
91. G. W. Arana, A. B. Santos, M. T. Laraia, S. McLeod-Bryant, M. D. Beale, L. J. Rames, J. M. Roberts, J. K. Dias and M. Molloy, *Am. J. Psychiatry*, 1995, **152**, 265–267.
92. K. Handwerker, *Harv. Rev. Psychiatry*, 2009, **17**, 184–205.
93. C. S. de Kloet, E. Vermetten, C. J. Heijnen, E. Geuze, E. G. Lentjes and H. G. Westenberg, *Psychoneuroendocrinology*, 2007, **32**, 215–226.
94. F. Duval, M. A. Crocq, M. S. Guillon, M. C. Mokrani, J. Monreal, P. Bailey and J. P. Macher, *Psychoneuroendocrinology*, 2004, **29**, 1281–1289.
95. T. Jovanovic, J. E. Phifer, K. Sicking, T. Weiss, S. D. Norrholm, B. Bradley and K. J. Ressler, *Psychoneuroendocrinology*, 2011, **36**(10), 1540.
96. D. A. Lewis and R. E. Smith, *J. Affect. Disord.*, 1983, **5**, 319–332.
97. D. Naber, P. Sand and B. Heigl, *Psychoneuroendocrinology*, 1996, **21**, 25–31.
98. E. S. Brown, T. Suppes, D. A. Khan and T. J. Carmody, 3rd, *J. Clin. Psychopharmacol.*, 2002, **22**, 55–61.
99. E. S. Brown, *Ann. NY Acad. Sci.*, 2009, **1179**, 41–55.
100. R. Caceda, B. Kinkead and C. B. Nemeroff, *Int. Rev. Neurobiol.*, 2007, **78**, 327–376.
101. M. P. Curran and D. M. Robinson, *Drugs*, 2009, **69**, 1853–1878.
102. V. Madaan and D. R. Wilson, *Drug News Perspect.*, 2009, **22**, 319–324.
103. I. Karagiannides and C. Pothoulakis, *Curr. Opin. Endocrinol. Diabetes Obes.*, 2009, **16**, 47–52.
104. P. E. Holtzheimer, 3rd and C. B. Nemeroff, *NeuroRx*, 2006, **3**, 42–56.
105. G. G. Nussdorfer and L. K. Malendowicz, *Peptides*, 1998, **19**, 949–968.
106. M. Hamke, I. Herpfer, K. Lieb, C. Wandelt and B. L. Fiebich, *Brain Res.*, 2006, **1102**, 135–144.



107. J. J. Rakofsky, P. E. Holtzheimer and C. B. Nemeroff, *Curr. Opin. Chem. Biol.*, 2009, **13**, 291–302.
108. M. S. Kramer, N. Cutler, J. Feighner, R. Shrivastava, J. Carman, J. J. Sramek, S. A. Reines, G. Liu, D. Snively, E. Wyatt-Knowles, J. J. Hale, S. G. Mills, M. MacCoss, C. J. Swain, T. Harrison, R. G. Hill, F. Hefti, E. M. Scolnick, M. A. Cascieri, G. G. Chicchi, S. Sadowski, A. R. Williams, L. Hewson, D. Smith, E. J. Carlson, R. J. Hargreaves and N. M. Rupniak, *Science*, 1998, **281**, 1640–1645.
109. M. S. Kramer, A. Winokur, J. Kelsey, S. H. Preskorn, A. J. Rothschild, D. Snively, K. Ghosh, W. A. Ball, S. A. Reines, D. Munjack, J. T. Apter, L. Cunningham, M. Kling, M. Bari, A. Getson and Y. Lee, *Neuropsychopharmacology*, 2004, **29**, 385–392.
110. M. Keller, S. Montgomery, W. Ball, M. Morrison, D. Snively, G. Liu, R. Hargreaves, J. Hietala, C. Lines, K. Beebe and S. Reines, *Biol. Psychiatry*, 2006, **59**, 216–223.
111. T. Furmark, L. Appel, A. Michelgard, K. Wahlstedt, F. Ahs, S. Zancan, E. Jacobsson, K. Flyckt, M. Grohp, M. Bergstrom, E. M. Pich, L. G. Nilsson, M. Bani, B. Langstrom and M. Fredrikson, *Biol. Psychiatry*, 2005, **58**, 132–142.
112. J. Tauscher, W. Kielbasa, S. Iyengar, F. Vandenhende, X. Peng, D. Mozley, D. R. Gehlert and G. Marek, *Eur. Neuropsychopharmacol.*, 2010, **20**, 80–87.
113. S. J. Mathew, M. Vythilingam, J. W. Murrough, C. A. Zarate, Jr, A. Feder, D. A. Luckenbaugh, B. Kinkead, M. K. Parides, D. G. Trist, M. S. Bani, P. U. Bettica, E. M. Ratti and D. S. Charney, *Eur. Neuropsychopharmacol.*, 2011, **21**, 221–229.
114. S. C. Huang and V. L. Korlipara, *Expert Opin. Ther. Pat.*, 2010, **20**, 1019–1045.
115. Y. Fujimura, F. Yasuno, A. Farris, J. S. Liow, M. Geraci, W. Drevets, D. S. Pine, S. Ghose, A. Lerner, R. Hargreaves, H. D. Burns, C. Morse, V. W. Pike and R. B. Innis, *Biol. Psychiatry*, 2009, **66**, 94–97.
116. M. A. Rosenkranz, *Psychol. Bull.*, 2007, **133**, 1007–1037.
117. T. J. Sajdyk, A. Shekhar and D. R. Gehlert, *Neuropeptides*, 2004, **38**, 225–234.
118. K. A. Paschos, S. Veletza and E. Chatzaki, *CNS Drugs*, 2009, **23**, 755–772.
119. S. P. Brothers and C. Wahlestedt, *EMBO Mol. Med.*, 2010, **2**, 429–439.
120. J. C. Morales-Medina, Y. Dumont and R. Quirion, *Brain Res.*, 2010, **1314**, 194–205.
121. T. Karl and H. Herzog, *Peptides*, 2007, **28**, 326–333.
122. J. P. Redrobe, Y. Dumont and R. Quirion, *Life Sci.*, 2002, **71**, 2921–2937.
123. S. P. Brothers, S. A. Saldanha, T. P. Spicer, M. Cameron, B. A. Mercer, P. Chase, P. McDonald, C. Wahlestedt and P. S. Hodder, *Mol. Pharmacol.*, 2010, **77**, 46–57.
124. A. Holmes, J. W. Kinney, C. C. Wrenn, Q. Li, R. J. Yang, L. Ma, J. Vishwanath, M. C. Saavedra, C. E. Innerfield, A. S. Jacoby, J. Shine,



- T. P. Iismaa and J. N. Crawley, *Neuropsychopharmacology*, 2003, **28**, 1031–1044.
125. K. Mitsukawa, X. Lu and T. Bartfai, *Cell. Mol. Life Sci.*, 2008, **65**, 1796–1805.
126. U. Sollenberg, T. Bartfai and U. Langel, *Neuropeptides*, 2005, **39**, 161–163.
127. K. Saar, A. M. Mazarati, R. Mahlapuu, G. Hallnemo, U. Soomets, K. Kilk, S. Hellberg, M. Pooga, B. R. Tolf, T. S. Shi, T. Hokfelt, C. Wasterlain, T. Bartfai and U. Langel, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 7136–7141.
128. T. Bartfai, X. Lu, H. Badie-Mahdavi, A. M. Barr, A. Mazarati, X. Y. Hua, T. Yaksh, G. Haberhauer, S. C. Ceide, L. Trembleau, L. Somogyi, L. Krock and J. Rebeck, Jr, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 10470–10475.
129. A. Floren, U. Sollenberg, L. Lundstrom, M. Zorko, J. Stojan, M. Budihna, M. Wheatley, N. P. Martin, K. Kilk, A. Mazarati, T. Bartfai, M. Lindgren and U. Langel, *Neuropeptides*, 2005, **39**, 547–558.
130. S. C. Ceide, L. Trembleau, G. Haberhauer, L. Somogyi, X. Lu, T. Bartfai and J. Rebeck, Jr, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 16727–16732.
131. R. M. Karlsson and A. Holmes, *Amino Acids*, 2006, **31**, 231–239.
132. J. D. Coplan, C. G. Abdallah, J. Kaufman, J. Gelernter, E. L. Smith, T. D. Perera, A. J. Dwork, A. Kaffman, J. M. Gorman, L. A. Rosenblum, M. J. Owens and C. B. Nemeroff, *Psychoneuroendocrinology*, 2011, **36**, 289–293.
133. D. van West, J. Del-Favero, Y. Aulchenko, P. Oswald, D. Souery, T. Forsgren, S. Sluijs, S. Bel-Kacem, R. Adolfsson, J. Mendlewicz, C. Van Duijn, D. Deboutte, C. Van Broeckhoven and S. Claes, *Mol. Psychiatry*, 2004, **9**, 287–292.
134. D. Mehta and E. B. Binder, *Neuropharmacology*, 2012, **62**(2), 654.
135. R. H. Derijk, *NeuroImmunoModulation*, 2009, **16**, 340–352.
136. E. B. Binder, R. G. Bradley, W. Liu, M. P. Epstein, T. C. Deveau, K. B. Mercer, Y. Tang, C. F. Gillespie, C. M. Heim, C. B. Nemeroff, A. C. Schwartz, J. F. Cubells and K. J. Ressler, *JAMA*, 2008, **299**, 1291–1305.
137. E. B. Binder, *Psychoneuroendocrinology*, 2009, **34**(1), S186–S195.
138. J. J. Siekierka, S. H. Hung, M. Poe, C. S. Lin and N. H. Sigal, *Nature*, 1989, **341**, 755–757.
139. G. M. Wozniak, J. Ruegg, G. A. Abel, U. Schmidt, F. Holsboer and T. Rein, *J. Biol. Chem.*, 2005, **280**, 4609–4616.
140. W. B. Denny, D. L. Valentine, P. D. Reynolds, D. F. Smith and J. G. Scammell, *Endocrinology*, 2000, **141**, 4107–4113.
141. H. Vermeer, B. I. Hendriks-Stegeman, B. van der Burg, S. C. van Buul-Offers and M. Jansen, *J. Clin. Endocrinol. Metab.*, 2003, **88**, 277–284.
142. G. Wiederrecht, S. Hung, H. K. Chan, A. Marcy, M. Martin, J. Calaycay, D. Boulton, N. Sigal, R. L. Kincaid and J. J. Siekierka, *J. Biol. Chem.*, 1992, **267**, 21753–21760.

## CHAPTER 12

# *Neuropeptide Receptors: Novel Therapeutic Targets for Depression and Anxiety Disorders*

SHIGEYUKI CHAKI\*<sup>a</sup> AND KOSUKE KANUMA<sup>b</sup>

<sup>a</sup> Molecular Function and Pharmacology Laboratories and; <sup>b</sup> Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama, Saitama 331-9530, Japan

\*Email: s.chaki@po.rd.taisho.co.jp

### 12.1 Introduction

Stress has been well recognized as the primary cause of depression and anxiety disorders. In the brain, short-chain neurotransmitters or neuromodulators, called neuropeptides, have been suggested to play a pivotal role in stress responses. Neuropeptides are generally localized in restricted brain regions and are processed from their immature form, or prepro-forms. The expression and secretion of neuropeptides change upon stress exposure so as to regulate hypothalamus-pituitary-adrenal (HPA) axis activity and monoaminergic neuronal activity. Thus, neuropeptides play important roles in stress responses through the regulation of these pathways, and the dysregulation of neuropeptide systems may cause stress-related disorders such as depression and anxiety. To date, receptors for numerous neuropeptides have been identified, many of which belong to the G-protein coupled receptor superfamily and have

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

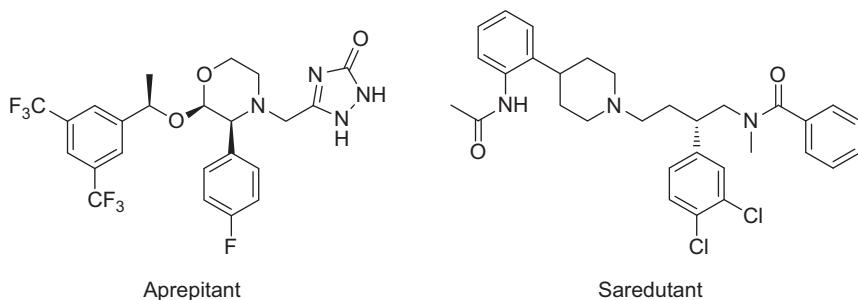
© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

subtypes with different localization and different physiological roles. The physiological roles of each receptor subtype are beginning to be elucidated and several neuropeptide receptors have been implicated in phenotypes associated with mood disorders in preclinical studies. Therefore, neuropeptide receptors provide an opportunity to develop novel treatments for depression and anxiety disorders, and many pharmaceutical companies have been actively pursuing the development of compounds that selectively act on a particular neuropeptide receptor subtype.

These activities have led to the development of several neuropeptide antagonists that have advanced to clinical trials for mood disorders including antagonists for the tachykinin receptors (NK1 and NK2), the corticotropin-releasing factor (CRF) receptor (CRF1), V1b (also called V3) receptor and  $\kappa$ -opioid receptor. Although both the NK1 receptor antagonist aprepitant and NK2 receptor antagonist saredutant (Figure 12.1) have been proven to be effective for the treatment of major depressive disorder in phase II studies,<sup>1</sup> Merck and Sanofi-aventis announced the discontinuation of the phase III clinical development of aprepitant and saredutant because of a lack of efficacy or development priority. Furthermore, Pfizer's CRF1 receptor antagonist, CP-316311, failed to demonstrate efficacy in a 6-week randomized, placebo-controlled trial using sertraline as an active comparator,<sup>2</sup> and this trial was terminated. Likewise, Sanofi-aventis has announced that further development of its V1b receptor antagonist, SSR149415, which was in phase II trials, has been halted. Despite these disappointing outcomes, neuropeptide receptors are still attracting interest as potentially novel approaches to the treatment of depression and anxiety. In addition to these well-studied neuropeptides, numerous neuropeptides and their receptors have been identified, and their roles in stress-related responses have been clarified using pharmacological tools as well as genetically engineered animals. Since most neuropeptides function in restricted brain areas and their functions may change depending on stress exposure, a particular neuropeptide receptor antagonist may be effective for specific types (or states) of patients with depression or anxiety disorders.

In this chapter, we will focus on neuropeptide systems, which have recently emerged as attractive and promising targets for the treatment of depression and



**Figure 12.1** NK receptor antagonists.

anxiety disorders. Of note, we will not discuss progress on tachykinin receptor antagonists because research in this area is no longer active in depression and anxiety following failures of clinical trials. However neuropeptide systems that regulate the HPA axis are included because some clinical trials are still active in this area.

## 12.2 Neuropeptide Receptors that Regulate Activity of the HPA Axis

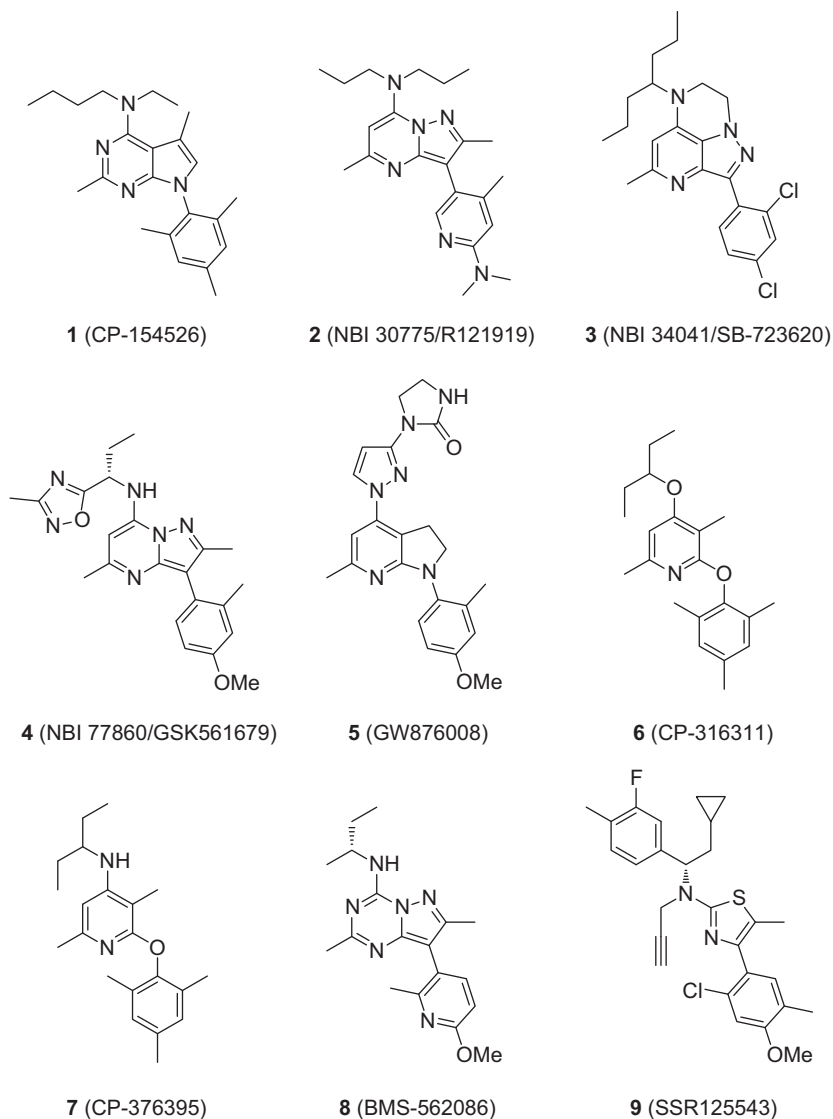
Abnormalities in hippocampus volume and HPA axis activity have been observed in patients with major depressive disorder, and treatment with antidepressants can normalize HPA activity. Both CRF and arginine vasopressin (AVP) are considered primary factors in the regulation of HPA axis activity,<sup>3</sup> and receptor subtypes for these neuropeptides, which may be involved in the regulation of HPA axis activity, have attracted much attention as potential targets for the treatment of depression and anxiety.

### 12.2.1 CRF1 Receptor

CRF, a 41-amino-acid peptide originally purified from ovine hypothalamus,<sup>4</sup> has been reported to mediate depressive and anxiety-like behaviour in rodents.<sup>5</sup> Several lines of evidence have indicated the involvement of CRF in psychiatric disorders, including depression and anxiety. Patients with major depressive disorder or post-traumatic stress disorder exhibit increased cerebrospinal fluid levels of CRF.<sup>6</sup> A blunted adrenocorticotropin (ACTH) response to the intravenous injection of CRF has been found in patients with depression, anorexia nervosa and post-traumatic stress disorder, suggesting pituitary CRF receptor down-regulation arising from chronic CRF hypersecretion.<sup>7,8</sup> *Post mortem* studies have shown increased CRF levels in the paraventricular nucleus (PVN) of the hypothalamus, locus coeruleus and frontal cortex,<sup>9–11</sup> and a characteristic increase in ACTH and cortisol response to the combined dexamethasone/CRH test is observed in patients with depressive disorders.<sup>12</sup>

Among the CRF receptors, which consist of two subtypes (CRF1 and CRF2), the role of the CRF1 receptor in depressive- and anxiety-like behaviours and the regulation of the HPA axis has been emphasized; to date, numerous small-molecule CRF1 receptor antagonists have been synthesized (*vide infra*). CRF1 receptor antagonists have exhibited antidepressant and anxiolytic effects in numerous rodent models.<sup>13</sup> Interestingly, most CRF1 receptor antagonists are more effective in models involving highly stressful conditions, consistent with the etiology of depression and anxiety. For example, we recently reported that R278995/CRA0450 induces an antidepressant-like activity in the learned helplessness paradigm and olfactory bulbectomy models.<sup>14</sup> Interestingly, CRA0450 displayed a significant effect in these models, even after a single administration, while fluvoxamine induced antidepressant

effects only following sub-chronic administration, suggesting that the CRF1 receptor antagonist has a faster onset.<sup>14</sup> The CRF1 receptor antagonist SSR125543, **9** (Figure 12.2), has been reported to exert its antidepressant effect independent of hippocampal neurogenesis, while increased (or renormalized) hippocampal neurogenesis (which usually requires a few weeks) is necessary for SSRIs to exert an antidepressant effect,<sup>15</sup> possibly explaining why CRF1 receptor antagonists may have a faster onset of action.



**Figure 12.2** CRF1 receptor antagonists.

To date, two clinical trials for major depressive disorder have been reported using R121919, **2**, and CP-316311, **6** (Figure 12.2). R121919, a CRF1 receptor antagonist, was tested in 20 patients with major depressive disorder in an open-label, dose-escalating trial conducted at the Max-Planck Institute, revealing a significant reduction in both depression and anxiety scores.<sup>16</sup> In a phase II trial, R121919 was found to be safe and well-tolerated, although it produced a slight elevation in liver enzyme levels.<sup>16</sup> However, CRF1 receptor antagonist, CP-316311, was reported to have no effect in depressed patients, while sertraline significantly reduced the HAM-D score in double-blind, placebo-controlled trials.<sup>2</sup> Moreover, another CRF1 receptor antagonist, ONO-2333Ms, was discontinued because of negative efficacy results in clinical trials (phase II) for major depression. With regard to anxiety disorders, the efficacy of NBI 34041, **3** (Figure 12.2), was tested using the Trier Social Stress Test (TSST).<sup>17</sup> In this trial, treatment with NBI 34041 attenuated increases in the plasma ACTH and cortisol concentrations induced by the TSST, indicating that the CRF1 receptor antagonist prevented stress-induced HPA axis activation. In addition, treatment with R317573 for 7 days was reported to be effective in a 7.5%-CO<sub>2</sub> model for anxiety in healthy volunteers<sup>18</sup> when used at a dose capable of altering regional cerebral glucose metabolism, as shown using [<sup>18</sup>F]fluoro-2-deoxy-D-glucose positron emission tomography.<sup>19</sup> However, the CRF1 receptor antagonist, pexacerfont, **8** (Figure 12.2), was not effective against generalized anxiety disorder in a multi-centre, randomized, double-blind, placebo-controlled trial, while escitalopram, an active comparator, was found to be effective.<sup>20</sup> The results of further clinical trials using CRF1 receptor antagonists may provide definitive conclusions as to their potential for the treatment of depression and anxiety disorders.

Since the first report of the small-molecule CRF1 receptor antagonist CP-154526 (**1**) (Figure 12.2),<sup>21</sup> a huge number of CRF1 receptor antagonists from a variety of chemical classes have appeared in literature. The first generation of CRF1 receptor antagonists, including CP-154526, were highly lipophilic and extremely insoluble in water, leading to a very long associated half-life and a high volume distribution. To circumvent this issue, a number of second-generation CRF1 receptor antagonists have been advanced in clinical trials for the treatment of depression and anxiety disorders.

### *NBI 30775/R121919 (2) (Neurocrine)*

Neurocrine discovered a potent CRF1 receptor antagonist, NBI 30775/R121919 (**2**) (Figure 12.2), in which the lipophilic pendant phenyl ring was substituted with a weakly basic pyridine.<sup>22</sup> NBI 30775/R121919 possesses a moderate lipophilicity with a log P value of 4.9, and its hydrochloride salt is readily soluble in water (> 10 mg/ml). Pharmacokinetic studies in rats showed that NBI 30775/R121919 had a high plasma clearance (CL = 112 ml/min/kg) and a large volume of distribution (Vd = 16.7 l/kg), resulting in a terminal

half-life of 1.7 h. NBI 30775/R121919 was orally bioavailable and penetrated the blood-brain barrier ( $F\% = 34\%$ ,  $B/P = 0.98$ ).

*NBI 34041/SB-723620 (3) (Neurocrine, GlaxoSmithKline)*

Neurocrine identified NBI 34041/SB-723620 (**3**) as a potent CRF1 receptor antagonist with a  $pK_i$  value of 8.3.<sup>23</sup> Restricting the side chain conformational flexibility of the pyrazolo[4,3-*b*]pyridine class ultimately led to new series of tricyclic antagonists, which were significantly less lipophilic because of the surprisingly basic nature of the core heterocycle.

*NBI 77860/GSK561679 (4), Verucerfont (Neurocrine, GlaxoSmithKline)*

As NBI 30775/R121919 possesses a high hepatic clearance, large distribution volumes and a high level of plasma protein binding, a main objective was to lower the lipophilicity of NBI 30775/R121919 ( $\log P = 4.9$ ) by introducing a heterocycle into one of the *N*-alkyl chains at the C(7)-position of the pyrazolo[1,5-*a*]pyrimidine core structure.<sup>24</sup> Upon the completion of a structure-activity relationship (SAR) study, Neurocrine and GlaxoSmithKline succeeded in replacing the alkyl chain with 1,2,4-oxadiazole in the top region, resulting in NBI 77860/GSK561679 (**4**). NBI 77860/GSK561679 proved to have a moderate clearance ( $CL = 14$  ml/min/kg) and distribution volume ( $V_d = 7.5$  l/kg). In addition, the human plasma protein binding level for NBI 77860/GSK561679 was 94%.

*GW876008, Emicerfont (5) (GlaxoSmithKline)*

GlaxoSmithKline attempted to replace the bis-heterocycle moiety present in the top region of the dihydropyrrole[2,3]pyridine template with a more hydrophilic non-aromatic heterocycle so as to form appropriate hydrogen-bond interactions with the amino acid residues Thr192 and Tyr195.<sup>25</sup> This exploration enabled the identification of a novel imidazolinone series of CRF1 receptor antagonists with a reduced overall lipophilicity. In particular, among the series, GW876008 (**5**) demonstrated appropriate *in vivo* pharmacokinetics and outstanding activity *in vivo* in an appropriate panel of animal models of anxiety in both rodents and primates.

*CP-316311 (6) (Pfizer)*

Pfizer's drug development activities have led to a new series of 2-aryloxy-4-alkoxy-pyridines as novel, selective and orally active antagonists of the CRF1 receptor based on structural modifications in the pyrrolopyrimidine core template.<sup>26</sup> The pyridine series represented one of the most attractive series because of its low molecular weight coupled with its excellent preclinical



efficacy. Furthermore, the compounds in this series proved to be safe and did not cause liver toxicity in five-day rat toxicology studies. SAR efforts finally identified a clinical compound, CP-316311 (**6**), that was advanced to a phase II clinical trial to test the ability of a CRF1 receptor antagonist to treat major depression or generalized anxiety disorder. Yet CP-316311 showed a significant positive food effect in dog and human after oral administration, possibly due to low solubility.<sup>2</sup>

### *CP-376395 (7) (Pfizer)*

After finding that CP-316311 exerted a significant positive food effect, Pfizer tried to improve the solubility and physicochemical properties of this compound.<sup>27</sup> CP-316311 was redesigned, and the oxygen at the alkoxy was replaced with an aminoalkyl, leading to the discovery of CP-376395 (**7**). CP-376395 has an increased basicity ( $pK_a = 6.9$ ) and solubility in simulated gastrointestinal fluid or at a low pH (0.1  $\mu\text{g/ml}$  at pH 7 and 5.4  $\text{mg/ml}$  at pH 2.4), leading to a significant impact on the reduction of fed-fasted food effects. However, the effect was only 2–3-fold in dogs and humans, compared with 10–20-fold for CP-316311.

### *BMS-562086, Pexacerfont (8) (Bristol-Myers Squibb)*

Bristol-Myers Squibb resolved the high lipophilicity and low water solubility issues by introducing a 2-methyl-6-methoxypyrid-3-yl group at the 8-position of the pyrazolotriazine core, leading to the discovery of BMS-562086 (**8**).<sup>28</sup> The solubilities were 16  $\mu\text{g/ml}$  in water (pH 7.4), 16.3  $\text{mg/ml}$  in 0.01 N HCl (pH 2.5), 300  $\text{mg/ml}$  in ethanol and 460  $\text{mg/ml}$  in acetone, and the HPLC log P value was 4.32. The good physicochemical properties confirmed a good maximal oral exposure ( $C_{\text{max}} = 1,260.8 \text{ nM}$ ), good oral bioavailability (40%) and an acceptable elimination half-life (13.5 h).

### *SSR125543 (9) (Sanofi-aventis)*

As shown for clinical compounds **1–8**, the structural features common to most CRF1 receptor antagonists include a core central heterocycle that contains a critical hydrogen bond acceptor, an out-of-plane pendant aromatic ring and a top side-chain filling a largely hydrophobic area of the receptor. These features have been previously classified into two topologies based on distinct structural features.<sup>29</sup> In the first topology, which contains the majority of compounds to have reached the clinical stage, the hydrogen bond acceptor and the pendant aryl substituent are separated by two atoms, while in the second topology, a single intervening atom is present. Sanofi-aventis developed a novel topology II template class, 2-aminothiazole derivatives, by optimizing a lead compound discovered during the random screening of several thousand compounds. This class is exemplified by SSR125543 (**9**), which exhibits high affinities for both native and recombinant human CRF1 receptors ( $K_i = 1$  and 2 nM, respectively).<sup>30,31</sup>

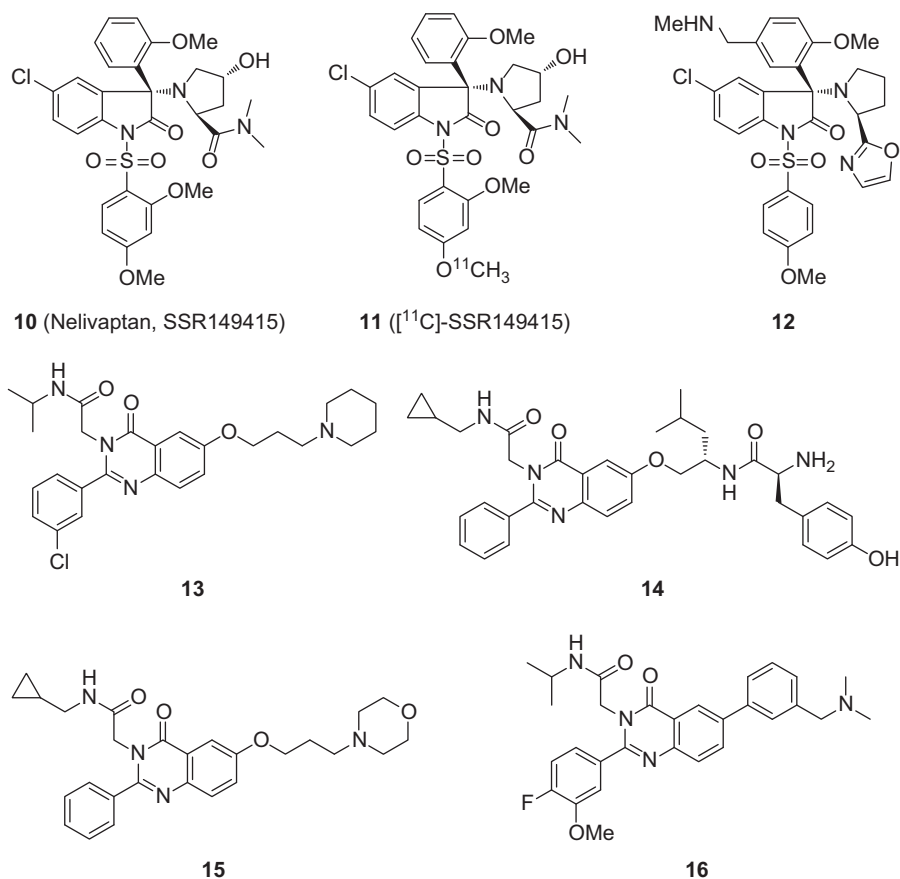
### 12.2.2 V1b (V3) Receptor

Arginine-vasopressin (AVP), a cyclic nonapeptide, is a principal factor in the regulation of ACTH release from the pituitary, together with CRF,<sup>3</sup> and has been reported to play an important role in mood regulation. Upon stress stimulation, AVP is released from the median eminence into the pituitary portal circulation, where it strongly potentiates the effects of CRF on the release of ACTH. Clinically, the plasma AVP levels are elevated in patients with major depression, compared with healthy controls.<sup>32,33</sup> In these studies, subjects with melancholic-type or anxious-retarded depression exhibited elevated plasma AVP levels.<sup>32,33</sup> The plasma AVP level is also reportedly correlated with cortisol levels during depression in a positive manner, particularly in suicide victims.<sup>34</sup> Moreover, the quantity of AVP-immunoreactive neurons and AVP mRNA in the PVN are increased during depression,<sup>35,36</sup> particularly in subjects in the melancholic subgroup.<sup>36</sup> The involvement of the AVP system in anxiety disorders has also been suggested. The plasma AVP levels of patients with PTSD are reportedly higher than those in both healthy controls and trauma controls,<sup>37</sup> and the AVP levels in the cerebrospinal fluid are increased in patients with obsessive-compulsive disorder.<sup>38</sup> AVP release is significantly correlated with anxiety symptom responses in healthy subjects challenged with an anxiogenic CCK-B agonist,<sup>39</sup> implicating AVP in these stress-related disorders. In contrast, the plasma AVP level is increased by the infusion of sodium lactate or hypertonic sodium chloride in both patients with panic disorders and healthy volunteers, although panic symptoms did not occur in healthy volunteers.<sup>40</sup>

The roles of AVP and CRF in HPA axis regulation may differ in acute and chronic stress responses. Although in acute stress responses CRF is the main activator of ACTH secretion, repeated stress markedly increases the proportion of AVP-containing neurons among the CRF neurons in the PVN<sup>41</sup> as well as pituitary V1b receptor expression.<sup>42</sup> Therefore, repeated stress exposure enhances the role of AVP in the regulation of HPA axis activity; thus, blockade of the AVP system may be a useful approach to treating conditions induced by chronic stress exposure, such as depression.

AVP exerts its effects *via* three receptor subtypes: V1a, V1b and V2. All these subtypes are G-protein coupled receptors (GPCRs).<sup>43</sup> Among them, the V1b receptor is mainly expressed in the pituitary and is thought to be involved in the regulation of HPA axis activity and emotional processes. To date, several series of small-molecule V1b receptor antagonists have been synthesized (*vide infra*). Among them, SSR149415 (Nelivaptan), **10** (Figure 12.3), which has a high affinity, selectivity and potent antagonist activity for the V1b receptor,<sup>44</sup> has been used as a pharmacological tool to characterize the role of the V1b receptor in depression and anxiety. SSR149415, **10**, has been reported to exert antidepressant and anxiolytic effects in animal models of depression and anxiety, with more pronounced effects in models involving stressful situations.<sup>45–47</sup> Distinct from a CRF1 receptor antagonist, SSR149415 exerted an antidepressant effect only following chronic (14 days) or sub-chronic (7 days)

treatment in an olfactory bulbectomy model,<sup>45,47</sup> suggesting the possibility that the CRF system may remain dominant in the regulation of HPA axis activity at the start of the administration of this compound. The antidepressant effects exerted by both the CRF1 receptor antagonist, SSR125543, **9**, (Figure 12.2), and V1b receptor antagonist, SSR149415, **10** (Figure 12.3), are reportedly independent of hippocampal neurogenesis, while, in contrast, hippocampal neurogenesis is necessary for monoamine-based antidepressants such as imipramine and fluoxetine.<sup>15</sup> In addition to the regulation of HPA axis activity, extra-hypothalamic nuclei (the lateral septum, central, basolateral and medial nucleus of the amygdala) are also reportedly involved in the antidepressant effect of SSR149415; among these nuclei, however, only the basolateral nucleus is involved in the anxiolytic effect.<sup>48,49</sup> In contrast, the pituitary V1b receptor (which is responsible for the regulation of the HPA axis) has been reported to play a role in the anxiolytic effect in the social interaction test,<sup>50</sup> but not in the antidepressant effect in the forced-swimming test.<sup>51</sup> Therefore, the



**Figure 12.3** V1b receptor antagonists.

neuroendocrine mechanisms underlying the antidepressant and anxiolytic effects of V1b receptor antagonists may differ.

Reports on clinical trials with V1b receptor antagonists are limited. SSR149415 (Sanofi-aventis) was tested in a phase II clinical trial for depression, but the progress of this compound has not been reported. ABT-436 (Abbott, structure not shown) is currently being tested in a phase I study for depression to assess its safety and pharmacology. In addition, a V1b receptor antagonist, which is a 2-(4-oxo-2-aryl-quinazolin-3(4*H*)-yl)acetamide derivative, has reportedly progressed to clinical development.<sup>52</sup>

The non-peptidic V1b receptor antagonists developed to date can be distinguished according to two chemotypes: oxindole and 4-quinazolinone.

### *Sanofi-aventis (Nelivaptan, SSR149415 (10))*

Sanofi-aventis discovered the first series of non-peptide V1b receptor antagonists based on an oxindole scaffold. Among them, nelivaptan (SSR149415, **10**, Figure 12.3) has been identified as a potent, selective and orally active V1b receptor antagonist.<sup>53</sup> Schönberger *et al.* developed a carbon-11 labelled version of SSR149415 (**11**) and elucidated its pharmacokinetics in non-human primates using positron emission tomography.<sup>54</sup>

### *Abbott (ABT-436)*

Abbott announced that ABT-436, the structure of which was not disclosed, entered a phase I clinical trial in December 2009. Recently, Abbott reported novel derivatives of SSR149415 (**10**) with improved pharmacokinetic properties.<sup>55</sup> SSR149415 (**10**) has a very short half-life and high plasma clearance in rats, consistent with a high intrinsic clearance in liver microsomes. The proline *N,N*-dimethylamide moiety was identified as a metabolic hot spot. Through incubation with human and rat liver microsomes, one of the *N*-methyl groups was found to be oxidized and cleaved. Therefore, they attempted to replace the *N,N*-dimethylamide with small heterocycles. Finally, oxazole **12** (Figure 12.3) was found to have moderate metabolic stability in human as well as in rat liver microsomes. Compound **12** exhibited an improved oral bioavailability, half-life and brain penetration, compared with SSR149415 (*F*% = 68%, *t*<sub>1/2</sub> = 3.3 h, *B/P* = 0.31). In the rat forced-swimming model, compound **12** exerted an antidepressant-like effect after the intra-peritoneal administration of 3 mg/kg.

### *Merck (formerly Organon)*

Merck (formerly Organon) characterized a novel series of quinazolin-4(3*H*)-ones as non-peptidic V1b receptor antagonists exemplified by compound **13** (Figure 12.3), which advanced to a phase I clinical trial. To identify a structurally distinct class of V1b receptor antagonists, a high-throughput screening (HTS) campaign from an in-house library was initiated, generating a hit

compound **14** (Figure 12.3) with an  $IC_{50}$  value of  $0.20\ \mu\text{M}$ .<sup>56</sup> From the perspective of “drug likeness”, compound **14** possessed less than suitable physicochemical properties ( $MW = 613$ ,  $FPSA = 148.48\ \text{\AA}$ ,  $clogP = 4.73$ , number of rotatable bonds = 17). Therefore, the pivotal objective of the hit-to-lead program was to generate a potent and tractable lead compound with improved physicochemical properties. The simplification of the tyrosine residue on the right-hand side established a significantly improved lead compound **15** (Figure 12.3), in terms of its physicochemical properties ( $MW = 477$ ,  $FPSA = 83.30\ \text{\AA}$ ,  $clogP = 2.45$ ). In addition, compound **15** demonstrated a high selectivity ( $> 1,000$ -fold) relative to the human  $V1a$ ,  $V2$  and  $OT$  receptors. Next, a lead optimization program of lead compound **15** was explored.<sup>52</sup> A drastic improvement in  $V1b$  affinity was achieved by substituting phenyl at the C(2)-position of quinazolinone and the optimization of the alkyl amide portion, leading to clinical compound **13** (Figure 12.3). Compound **13** showed an excellent selectivity against  $V1a$ ,  $V2$  and  $OT$  receptor subtypes and against a broad panel of unrelated targets such as GPCRs, ion channels, transporters and enzymes (Novascreen). Also, compound **13** exhibited promising pharmacokinetic profiles in rats. A moderate clearance and a high distribution volume resulted in a long elimination half-life of 3.8 h. Compound **13** had good oral bioavailability ( $F\% = 53\%$ ) and no CYP inhibition profile. Given its potent antagonism and suitable PK profiles, compound **13** was further profiled in an *in vivo* model of HPA hyperactivity, where it was shown to antagonize the effects of dDAVP on elevating ACTH levels in rats when administered at an oral dose of 5 mg/kg. Compound **13** exhibited a promising pK profile with good oral exposure and a half-life suitable for once daily dosing in human. Moreover, no deviation from dose-proportionality was observed over a dose range of from 1 to 150 mg. In addition to this series, Merck reported the replacement of the propyloxy spacer with an aryl substituent to reduce the number of rotatable bonds, leading to compound **16** (Figure 12.3) with a low nanomolar affinity for the  $V1b$  receptor, good selectivity against related receptors  $V1a$ ,  $V2$  and  $OT$ , a good pharmacokinetic profile and activity in a mechanistic model of HPA dysfunction.<sup>57</sup>

## 12.3 Neuropeptide Receptors that Regulate Reward Activity

Given that clinical depression is marked by anhedonia (diminished interest or pleasure), dysfunction of the brain reward pathway is thought to contribute to the pathophysiology of depression. Since the nucleus accumbens is the centre of reward, anhedonia is hypothesized to arise if the function of the nucleus accumbens is hampered. Indeed, stress, drug exposure and drug withdrawal, all of which produce a depressive-phenotype in humans, reportedly alter various functions within the nucleus accumbens leading to inhibited dopaminergic activity in the region. Neuropeptide receptors regulating dopaminergic activity in the nucleus accumbens have thus received much attention.

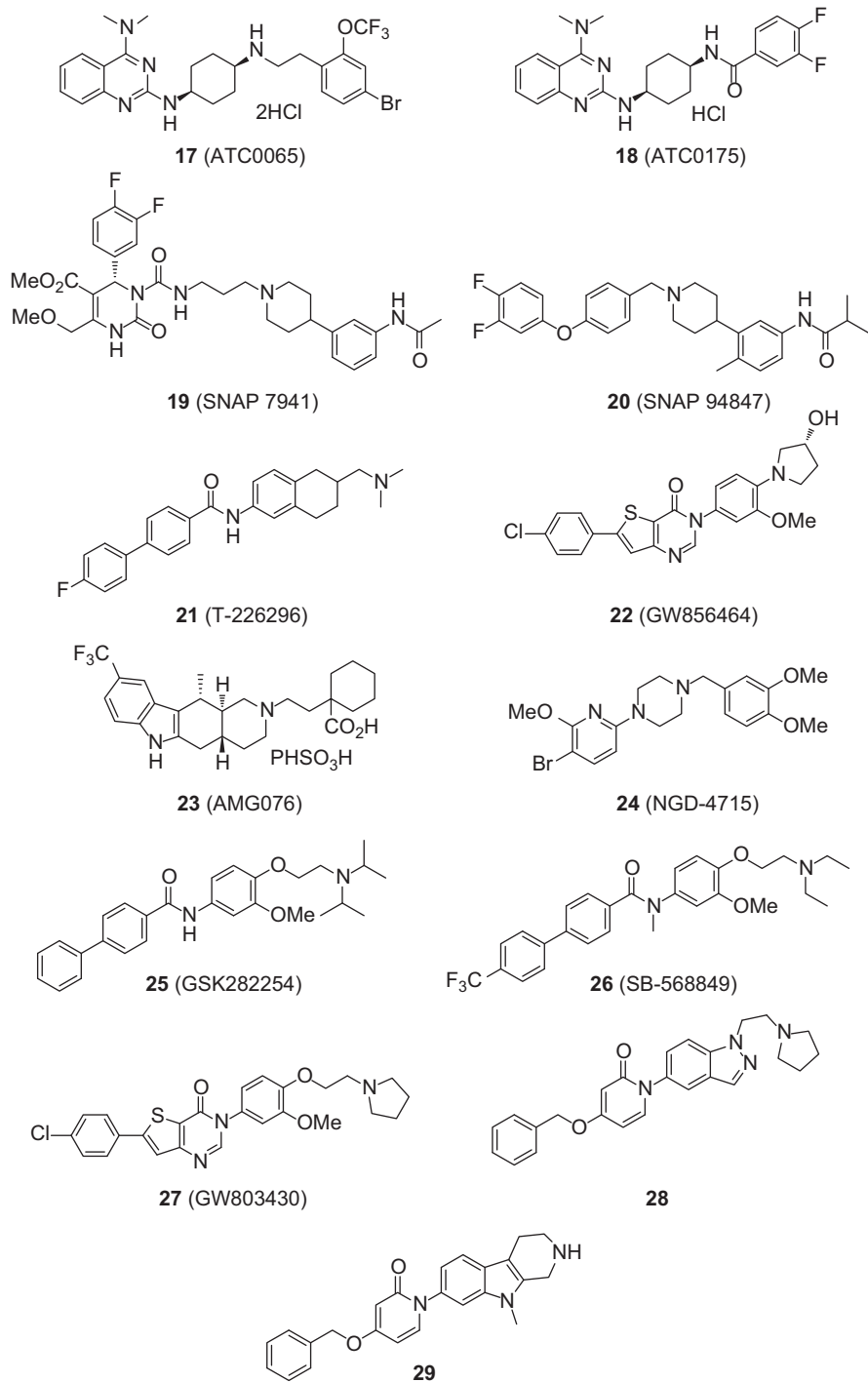
### 12.3.1 MCH1 Receptor

Melanin-concentrating hormone (MCH), a cyclic 19-amino acid peptide, produced predominantly by neurons in the lateral hypothalamus and zona incerta with extensive projections throughout the brain, reportedly mediates numerous physiological effects including increased food intake and the production of depressive and anxiety-like behaviors.<sup>58</sup> Two receptor subtypes have been reported, the MCH1 receptor and the MCH2 receptor, both of which belong to the GPCR superfamily.<sup>58</sup> Of these, the MCH1 receptor has been suggested to mediate most of the physiological effects of MCH, including the regulation of feeding behaviour and emotional states.

The MCH1 receptor is densely expressed in the nucleus accumbens shell, a brain region involving motivation and reward, and the local injection of MCH into the nucleus accumbens shell reportedly results in a depressive phenotype in the forced-swimming test, while the local injection of an MCH1 receptor antagonist produces antidepressant effects.<sup>59</sup> This result is consistent with the finding that knockout mice lacking the MCH1 receptor exhibited enhanced mesolimbic dopaminergic activity<sup>60</sup> and an antidepressant-like phenotype,<sup>61</sup> suggesting that increased reward activity may be responsible for the antidepressant effect induced by the blockade of the MCH1 receptor. In addition, the MCH1 receptor is expressed in monoaminergic nuclei including the dorsal raphe nucleus and locus coeruleus, and the injection of MCH into the dorsal raphe nucleus produced depressive behaviour in the forced-swimming test, while the injection of anti-MCH antibody into the same region produced an antidepressant effect.<sup>62</sup> Moreover, the MCH1 receptor is reportedly distributed densely in the PVN of the hypothalamus, where it has a stimulatory effect on HPA axis activity through CRF secretion;<sup>63</sup> this mechanism may be involved in the depressive and anxiety-related effects of MCH. Indeed, the intra-cerebroventricular injection of MCH reportedly increases the plasma ACTH levels, while this increase is prevented by an MCH1 receptor antagonist.<sup>64</sup>

To date, a variety of non-peptide MCH1 receptor antagonists have been reported as described below. Some MCH1 receptor antagonists including ATC0065 (**17**), ATC0175 (**18**), SNAP 7941 (**19**) and SNAP 94847 (**20**) (Figure 12.4) reportedly exhibit antidepressant and anxiolytic effects in several animal models of depression and anxiety.<sup>64–67</sup> Interestingly, the chronic administration of SNAP 94847 increased progenitor cell proliferation in the dentate gyrus, but the suppression of hippocampal neurogenesis by X-irradiation did not alter the antidepressant effect of SNAP 94847 on novelty-suppressed feeding;<sup>67</sup> thus, the mechanism responsible for the effects of SNAP 94847 may be distinct from that of SSRIs but similar to those of CRF1 receptor antagonists and V1b receptor antagonists. As observed using a CRF receptor antagonist, SNAP 94847 reversed the decrease in sucrose intake with a faster onset of action (<1 week), compared with the SSRI citalopram (<2 weeks), in a chronic mild stress paradigm.<sup>64</sup>

Since the discovery of the pioneering MCH1 receptor antagonist T-226296, a (–)-enantiomer of compound **21**, by Takeda in 2001,<sup>68</sup> a huge number of



**Figure 12.4** MCH1 receptor antagonists.



drug-discovery programs have focused on the identification of small-molecule MCH1 receptor antagonists for the treatment of obesity and/or mood disorders. Nevertheless, only five compounds, GW856464 (**22**, GlaxoSmithKline), AMG076 (**23**, Amgen), NGD-4715 (**24**, Neurogen), BMS-830216 (undisclosed structure, Bristol-Myers Squibb) and ALB-127158(a) (undisclosed structure, AMRI), have progressed to clinical development for the treatment of obesity. Beyond the common challenges in drug design related to ADME and safety profiles, a cardiovascular risk involving human ether-a-go-go related gene (hERG) binding and the potential for subsequent drug-induced QTc prolongation has been a major hurdle for a significant number of MCH1 receptor research programs.<sup>69</sup> Phase I clinical trials for GW856464, AMG076 and NGD-4715 have already been terminated because of side-effects. In particular, while Neurogen reported that no serious adverse events were observed, vivid dreams and awakenings were reported by 50% of NGD-4715-treated patients during a one-week treatment period. Although there are encouraging pre-clinical data predictive of antidepressant and anxiolytic efficacy, most MCH1 receptor antagonists have been tested in clinical trials for obesity.

### *GlaxoSmithKline (GW856464)*

GlaxoSmithKline developed thienopyrimidone MCH1 receptor antagonists exemplified by GW856464, **22** (Figure 12.4), which progressed to a clinical trial in 2004 but has already been terminated. GlaxoSmithKline initiated an MCH1 receptor program *via* an HTS of the corporate compound collection, resulting in the identification of the biphenyl carboxamide GSK282254 **25** with a  $pK_i$  of 7.5 in an MCH1 receptor binding assay.<sup>70</sup> GSK282254 showed a significant affinity for the 5-HT<sub>2C</sub> receptor, with a  $pK_i$  of 6.8, a moderate *in vitro* clearance of 9 ml/min/g in rat liver microsomes and a low solubility of 4  $\mu$ g/ml at a physiological pH, resulting in low oral bioavailability. An optimization campaign around the terminal biphenyl, the central aniline ring, the alkylamino side chain and the amide linker led to the identification of SB-568849, **26**, which exhibited effective MCH1 receptor antagonism, a low *in vivo* clearance, a good brain-blood ratio and oral bioavailability in rats ( $CL_b$  = 16 ml/min/kg, B/P = 1, F% = 30%). Furthermore, GlaxoSmithKline succeeded in improving the MCH1 receptor antagonist activities and brain penetration by introducing the molecule to a conformational constraint against the carbonyl moiety, leading to GW803430, **27** (B/P = 6).<sup>71,72</sup> GW803430 demonstrated a significant dose-dependent weight reduction relative to vehicle controls but a liability against the hERG. Finally, GlaxoSmithKline overcame the hERG issue by attenuating the basicity of the side chain, leading to the discovery of the clinical compound GW856464 (**22**).

### *AMRI (formerly Albany Molecular Research)*

AMRI initiated a phase I study of ALB-127158(a); in May 2011 at the 18th European Congress on Obesity, the company announced that ALB-127158(a)

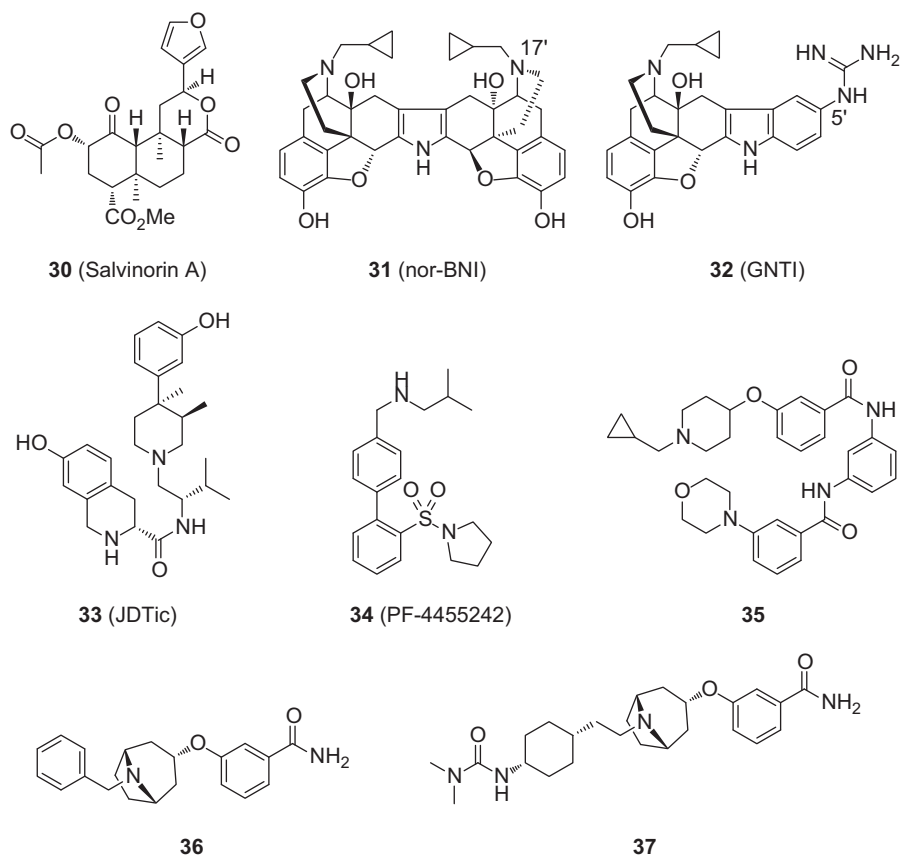
was well tolerated at the doses tested, with preliminary evidence suggesting efficacy. AMRI described a series of 5-(pyridinon-1-yl)indazoles, exemplified by compound **28** (Figure 12.4), with a high affinity for the MCH1 receptor ( $K_i = 2.6 \text{ nM}$ ).<sup>73</sup> Compound **28** showed a statistically significant reduction in body weight (2.8%) at a once-daily dose of 60 mg/kg. However, this weight loss was significantly increased (6.0%) when a twice-daily dosing of 30 mg/kg was used, suggesting that the pharmacokinetic profile of this compound could be further improved. Therefore, an optimization campaign was initiated to explore the SAR around compound **28** to improve the pharmacokinetic profile of this series.<sup>74,75</sup> A conformational constraint between the indazole ring and basic amine led to the identification of a new tetrahydrocarbolines scaffold as a potent MCH1 receptor antagonist. Compound **29** (Figure 12.4) was found to be a highly selective MCH1 receptor antagonist, with no significant off-target activity, amongst a panel of 88 GPCRs and transporters. Furthermore, compound **29** boosted the oral exposure, the brain concentration and the effect on reducing body weight (9.1% reduction after dosing using a once-daily dose of 30 mg/kg).

### 12.3.2 $\kappa$ Opioid Receptor

The endogenous opioid systems, consisting of three families of neuropeptides (endorphins, enkephalins and dynorphins), are thought to be involved in emotional and behavioural responses to stress. The dynorphin family has six peptides of varying lengths formed from the precursor prodynorphin.<sup>76</sup> Among opioid receptors ( $\mu$ ,  $\delta$  and  $\kappa$  receptors), dynorphins activate the  $\kappa$  opioid receptor (KOR). The activation of KOR reportedly leads to dysphoria (defined as an unpleasant or aversive state) in humans,<sup>77</sup> and depressive-like behaviours in rodents.<sup>78</sup> Moreover, salvinorin A, **30** (Figure 12.5), a KOR agonist, has been reported to produce anxiogenic and unpleasant effects in humans that deter repeated or compulsive use.<sup>79</sup> Thus, the activation of KOR may have etiological roles in depression and anxiety.

KOR antagonists such as nor-BNI, **31** (Figure 12.5), ANTI and DIPPA reportedly exhibit antidepressant effects in animal models of depression (forced-swimming test, social defeat stress) and anxiety (novelty-suppressed feeding, defensive burying test).<sup>80–82</sup> Recently, a novel and small-molecule KOR antagonist, PF-4455242, **34** (Figure 12.5), has been reported; this compound exerts an antidepressant activity in the forced-swimming test and in response to social defeat stress at doses that occupy the central KOR and attenuate a KOR agonist-induced plasma prolactin level,<sup>83</sup> demonstrating that this compound exerts antidepressant effects by blocking central KOR.

KOR is widely expressed throughout the brain, and KOR expressed in the nucleus accumbens may be involved in depression. Thus, exposure to learned helplessness, immobilization stress and forced-swim stress increases the levels of dynorphins immunoreactivity in the NAc,<sup>84</sup> and the injection of KOR agonists in this area causes conditioned place aversions,<sup>85</sup> while the injection of



**Figure 12.5**  $\kappa$  opioid receptor antagonists.

KOR antagonists into the nucleus accumbens produces antidepressant effects in the learned helplessness paradigm.<sup>84</sup> GABAergic projection neurons in the nucleus accumbens receive inputs from ventral tegmental dopamine neurons that express dynorphin. Dynorphin serves as a negative feedback mechanism by acting on pre-synaptic KOR to inhibit dopamine neuronal function.<sup>86</sup> Indeed, both the systemic and intra-accumbal injection of a KOR agonist reduced dopamine release in the nucleus accumbens, while the injection of a KOR antagonist increased dopamine release.<sup>87</sup> Thus, given that the dopamine system in the nucleus accumbens plays a major role in motivation and reward and could contribute to the anhedonia observed in depressed patients, an increase in mesolimbic dopaminergic activity may be responsible for the antidepressant actions of KOR antagonists. This assumption is supported by a recent finding showing that a KOR antagonist attenuates depressive behaviours associated with cocaine withdrawal, which is characterized by the dysregulation of brain reward systems.<sup>88</sup> In addition to the nucleus accumbens, the

hippocampal nuclei and the piriform cortex have been thought to be potential sites of action of KOR antagonists, because the local injection of a KOR antagonist produces antidepressant effects.<sup>84,89</sup>

While many agonists of KOR have been identified, very few antagonists have appeared to date. Applying the message-address concept,<sup>90</sup> Portoghese developed the first selective non-peptide KOR antagonist, nor-binaltorphimine (nor-BNI, **31**) (Figure 12.5).<sup>91</sup> *N*-17' basic nitrogen has an important role in the "address" component and contributes to KOR selectivity by interacting with an acidic residue specific to the receptor. Further refinements of this concept led to the discovery of C5'-guanidinylnaltrindole (GNTI, **32**), which enhanced the potency and selectivity of KOR antagonism, compared with nor-BNI.<sup>92</sup> In 2001, Research Triangle Institute identified a *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonists without a morphine-like structure, exemplified by JDTic, **33** (Figure 12.5).<sup>93</sup> JDTic exhibited minimal KOR selectivity against the  $\mu$  opioid receptor in binding assays but excellent selectivity and KOR antagonist potency in functional assays.

Thereafter, several affiliations initiated research to discover selective KOR antagonists for potential therapeutic targets. As a result, Pfizer and Eli Lilly succeeded in developing the clinical compounds, PF-4455242, **34** (Figure 12.5), and LY-2456302 (undisclosed structure). In 2009, Pfizer identified a potent and selective KOR antagonist, PF-4455242, **34**, which progressed to a clinical trial for the potential oral treatment of bipolar disorder.<sup>94</sup> However, by September 2010, the program had been discontinued because of toxicology findings in animals exposed to PF-4455242 for three months. In 2010, Eli Lilly advanced LY-2456302 to a phase I study for the potential oral treatment of alcohol dependence to measure the occupancy of brain KOR after a single oral dose of LY-2456302.

Recently, AstraZeneca disclosed a new series of 8-azabicyclo[3.2.1]octan-3-yloxy-benzamides as KOR antagonists.<sup>95,96</sup> Initially, they identified a series of potent and highly selective bis-amide alkoxy piperidines represented by compound **35** (Figure 12.5) from an HTS of their corporate compound collection ( $IC_{50}$  for  $\kappa$ ,  $\mu$  and  $\delta$  = 77 nM, >30,000 nM and >30,000 nM, respectively). However, compound **35** exhibited a poor permeability in an MDCK-MDR1 *in vitro* assay, resulting in minimal brain exposure. The poor permeability was thought to be due to the high molecular weight. They initiated a hit-to-lead campaign in an attempt to identify the most critical pharmacophore structure, leading to 8-azabicyclo[3.2.1]octane, **36** (Figure 12.5), while still maintaining selectivity *versus*  $\mu$  and  $\delta$  receptors ( $IC_{50}$  for  $\kappa$ ,  $\mu$  and  $\delta$  = 286 nM, 10,700 nM and 27,400 nM, respectively). The lead compound **36** was associated with a significant hERG liability and a high *in vitro* P-glycoprotein (P-gp) efflux. To address these issues, a SAR study was initiated, resulting in compound **37** (Figure 12.5). Compound **37** exhibited good KOR antagonism and selectivity *versus*  $\mu$ ,  $\delta$  and hERG. However, the P-gp efflux ratio of compound **37** was moderate, leading to a poor brain exposure.

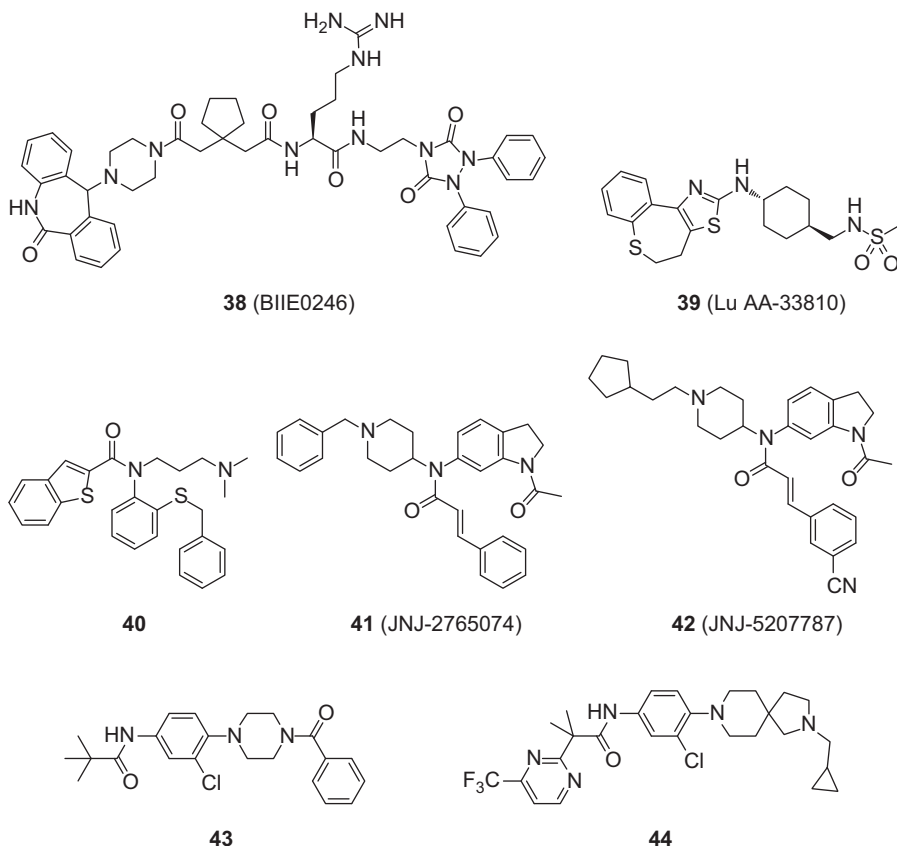
## 12.4 Neuropeptide Systems that Regulate Feeding

### 12.4.1 Neuropeptide Y

Neuropeptide Y (NPY), a highly conserved 36-amino acid peptide, is the most abundant peptide in the central nervous system and is expressed in numerous brain areas, including the ventral and dorsal striatum, limbic structures and brain stem (the locus coeruleus). NPY plays an important role in numerous physiological processes including food intake, cognition and pain perception.

In human studies, relative reductions in NPY peptide or NPY mRNA expression have been associated with major depressive disorder. Depressed patients showed lower NPY levels in plasma than control subjects.<sup>97,98</sup> Moreover, robust reductions in the NPY levels were observed in the cerebrospinal fluid of medication-free subjects with treatment-refractory depression,<sup>99</sup> while the plasma NPY levels did not differ among subjects with panic disorders, subjects with social phobia or control subjects.<sup>100</sup> In a *post mortem* study, NPY-immunoreactivity was significantly lower in the frontal cortex and caudate nucleus from suicide victims than that in age-matched controls, with an even more robust reduction seen in a subgroup of suicide victims with a history of depression.<sup>101</sup> In contrast, antidepressant treatment (citalopram) increased the NPY levels in the cerebrospinal fluid, and this change was associated with a change in the HAM-D score.<sup>102</sup> Likewise, an increase in the NPY levels in the cerebrospinal fluid was observed following electroconvulsive therapy (ECT); this increase paralleled the clinical recovery.<sup>103</sup> Alterations in the NPY level have also been linked to depression and anxiety in animal studies. Genetic animal models of depression, such as the Flinders-Sensitive Line and Fawn Hooded rats showed lower NPY levels in the hippocampus, compared with the levels in the control group.<sup>104,105</sup> NPY levels (mRNA or immunoreactivity) in the hippocampus were decreased in several animal models of depression,<sup>106,107</sup> while antidepressant treatments (antidepressants, mood stabilizers, wheel running, ECT) increased the NPY levels in the hippocampus.<sup>108</sup> Thus, the NPY expression level may be related to depressive behaviour and antidepressant effects.

NPY exerts its effects through five receptor subtypes (Y1, Y2, Y4, Y5 and Y6). Of these subtypes, the main receptor subtypes mediating depressive and anxiety-like behaviours in rodents are the Y1, Y2 and Y5 receptors. Y1 receptor agonists mimic the anxiolytic action of NPY,<sup>109</sup> while Y1 receptor antagonists increase anxiety.<sup>110</sup> On the other hand, the Y2 receptor acts as a pre-synaptic NPY auto-receptor; thus, the blockade of this receptor increases the release and synthesis of NPY. BIIE0246, **38** (Figure 12.6), a Y2 receptor antagonist, has been reported to exert antidepressant and anxiolytic effects in animal models,<sup>111,112</sup> and mice lacking the Y2 receptor exhibited antidepressant-like and anxiolytic-like phenotypes.<sup>113</sup> These results indicate that the blockade of the pre-synaptic Y2 receptor enhances the release of NPY, resulting in the stimulation of a post-synaptic NPY receptor, presumably the Y1 receptor, and the induction of anxiolytic effects. In addition to the Y2



**Figure 12.6** NPY receptor antagonists.

receptor, the involvement of the Y4 receptor in depression and anxiety has been suggested, based on findings that knockout mice lacking the Y4 receptor display anxiolytic and antidepressant-like phenotypes; and similar to the Y2 receptor, the Y4 receptor is also localized pre-synaptically on the terminal and may possibly be involved in this action.<sup>114</sup> Recently, pharmacological data using a small molecule Y5 receptor antagonist, Lu AA33810, **39** (Figure 12.6), have been reported.<sup>115,116</sup> Lu AA33810 exhibits antidepressant and anxiolytic effects in some animal models, and blocks Y5 receptor agonist-induced plasma ACTH and corticosterone secretion. Given the extensive co-expression of the Y5 receptor with CRF or AVP-positive neurons in the PVN,<sup>117</sup> Y5 receptor blockade may exert its antidepressant and anxiolytic effects by regulating HPA axis activity.

Small-molecule NPY Y2 receptor antagonists have proved elusive until very recently; hence, the therapeutic potential of this receptor has remained relatively unexplored to date.

Boehringer Ingelheim characterized the pseudo-peptide BIIE0246, **38** (Figure 12.6), as a potent Y2 receptor antagonist that has virtually no affinity for Y1, Y4 or Y5 receptors.<sup>118</sup> However, the therapeutic potential of BIIE0246 and its analogues may be limited due to their peptidic nature and high molecular weight.

The first non-peptidic Y2 receptor ligands were disclosed by Bristol-Myers Squibb, which identified a series of benzothiophenes from an HTS, as exemplified by compound **40** (Figure 12.6); however, no details of their functional activity were provided.<sup>119</sup>

Johnson & Johnson discovered a series of novel small-molecule Y2 receptor antagonists, exemplified by the piperidinyllindoline cinnamide JNJ-2765074, **41** (Figure 12.6), from an HTS of their corporate compound collection.<sup>120</sup> Through a medicinal chemistry program for the series, JNJ-5207787, **42** (Figure 12.6), was identified as the most potent Y2 receptor antagonist. This compound was also found to be more than 100-fold selective *versus* human Y1, Y4 and Y5 receptors as evaluated using radioligand binding.

Recently, GlaxoSmithKline identified a novel small Y2 receptor antagonist, compound **43** (Figure 12.6), as a promising initial HTS hit.<sup>121</sup> Compound **43** was selective over both Y1 and Y5 receptors and showed moderate aqueous solubility and good *in vitro* metabolic stability (solubility = 46 µg/ml, Clint = 0.5 and 1.6 for human and rat, respectively). Therefore, an optimization campaign was initiated to explore the SAR around compound **43** to improve antagonist activity and aqueous solubility, leading to the development of a selective, soluble Y2 receptor antagonist, compound **44** (Figure 12.6), with good central nervous system exposure (solubility = 225 µg/ml, B/P = 2.3).<sup>122</sup>

While no non-peptide Y1 receptor agonists have been published to date, several potent non-peptide Y1 receptor antagonists have been identified as potential treatment for obesity.<sup>123</sup>

## 12.4.2 Other Neuropeptide Receptors

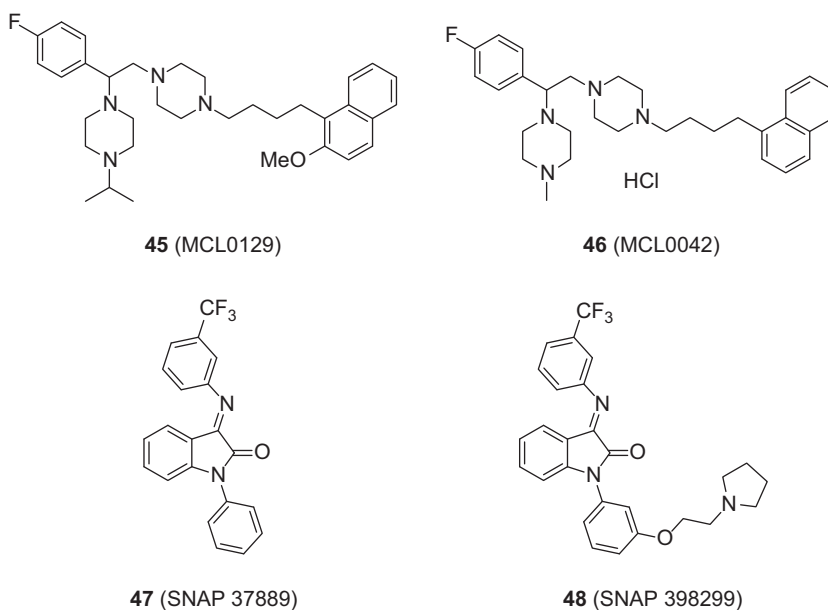
Melanocortins (ACTH and  $\alpha$ -,  $\beta$ - and  $\gamma$ -melanocyte-stimulating hormone [ $\alpha$ -,  $\beta$ - and  $\gamma$ -MSH]) are derived from proopiomelanocortin (POMC) through enzymatic processing, and participate in a wide range of physiological functions. Melanocortins have five receptor subtypes (MC1–MC5); among them, MC4 receptors are attracting interest for the treatment of depression and anxiety. The cyclic peptide antagonist MT II, an MC4 receptor agonist, potently induces grooming in rats, while the peptidic dual MC3/MC4 receptor antagonist SHU9119 has been reported to attenuate both MT II- and novelty-induced excessive grooming.<sup>124</sup> Moreover, MT II produces anxiogenic-like behaviour in the social interaction test,<sup>125</sup> suggesting that the activation of the MC4 receptor may cause anxiety-like behaviours. The MC4 receptor has been thought to produce these stress-related behaviours *via* the activation of HPA axis activity. Thus, the intra-cerebroventricular injection of MT II increases plasma corticosterone levels, which is prevented by a CRF receptor



antagonist,<sup>126</sup> indicating that the MC4 receptor activates the activity of the HPA axis by stimulating CRF expression and release in the PVN of the hypothalamus.

Non-peptide MC4 receptor antagonists, such as MCL0129, **45**, and MCL0042, **46** (Figure 12.7), reportedly exhibit antidepressant and anxiolytic effects in several animal models of depression.<sup>127,128</sup> Interestingly, the acute administration of MCL0129 exerted antidepressant effects in the learned helplessness paradigm, indicating a faster onset of action. Clinical evidence for MC4 receptor antagonists is not yet available.

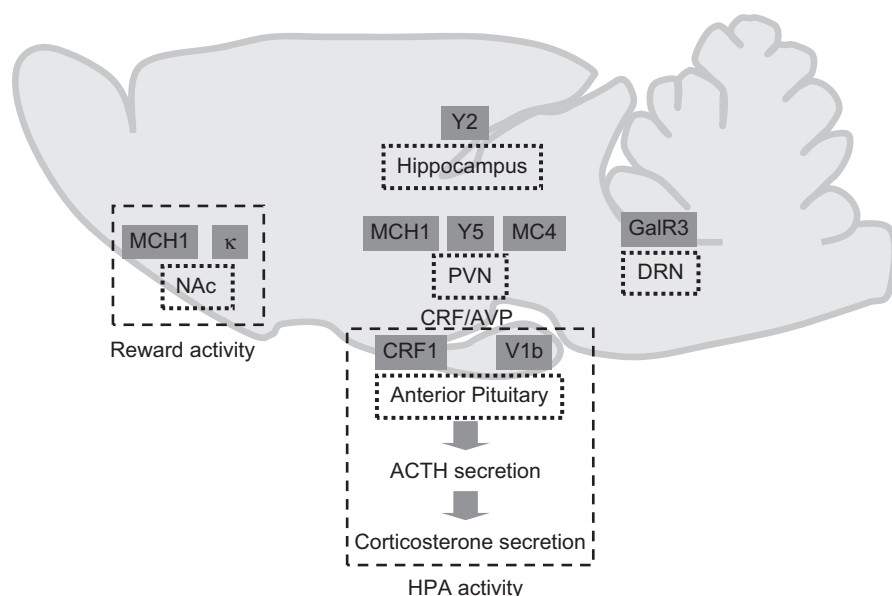
Galanin is a 29-amino-acid (in humans) or a 30-amino-acid neuropeptide detected in the serotonin-producing neurons of the dorsal raphe nucleus and in brainstem norepinephrine-producing neurons of the locus coeruleus.<sup>129</sup> The injection of galanin into the ventral tegmental area, but not into the lateral ventricles, midbrain reticular formation or hypothalamus, significantly increased immobility in the forced-swimming test, while galantide (M15), a galanin receptor antagonist, exerted an antidepressant effect.<sup>130</sup> In contrast, galanin has been reported to fail to alter depressive-like behaviour in the tail-suspension test in C57BL/6J mice,<sup>131</sup> indicating that the depressive behaviour induced by galanin may also be task- and strain-dependent. To date, three subtypes (GalR1, GalR2, GalR3) have been identified; among the galanin receptor subtypes, pharmacological studies using non-peptide GalR3 antagonists (SNAP 37889, **47**, SNAP 398299, **48** (Figure 12.7)) have suggested the involvement of GalR3 in depression and anxiety. Thus, these selective and



**Figure 12.7** MC4 receptor antagonists and GalR3 antagonists.

potent GalR3 antagonists have been reported to exhibit anxiolytic (social interaction, stress-induced hyperthermia, separation-induced vocalization, punish drinking) and antidepressant effects (forced-swimming test).<sup>132</sup> Also, galanin inhibited the firing of dorsal raphe serotonin neurons, and this inhibition was blocked by a GalR3 antagonist. Therefore, galanin may exert a depressive phenotype by inhibiting serotonergic activity, while GalR3 antagonists may exert antidepressant effects by attenuating the tonic inhibition of galanin on serotonergic transmission. Moreover, another group has reported that a GalR3 antagonist patented by Lundbeck, which differs from the above-mentioned SNAP compounds, exhibited antidepressant effects in the forced-swimming test and tail-suspension test, but no effects in the elevated plus-maze.<sup>133</sup>

The relationship among neuropeptides and their receptors described in this chapter is summarized in Figure 12.8.



**Figure 12.8** The relationships among neuropeptides and their receptors. CRF and AVP are produced in the PVN, and act on CRF1 and V1b receptors in the anterior pituitary, respectively, to regulate ACTH secretion. The synthesis and secretion of CRF and AVP in the PVN are regulated by melanocortins, neuropeptide Y and MCH through MC4, Y5 and MCH1 receptors, respectively. MCH1 and  $\kappa$  receptors are densely distributed in the nucleus accumbens shell where they regulate dopaminergic activity. GalR3 is expressed in the DRN where it regulates serotonergic activity. PVN: paraventricular nucleus; DRN: dorsal raphe nucleus; NAc: nucleus accumbens; HPA: hypothalamus-pituitary-adrenal; CRF: corticotropin-releasing factor; AVP: arginine-vasopressin.

## 12.5 Conclusions and Future Direction

The disappointing clinical outcomes of the NK1 antagonist aprepitant (phase III) and the CRF1 antagonist CP-316311 (phase II) for the treatment of major depressive disorders may have a large impact on the strategies of pharmaceutical companies regarding the development of agents acting on neuropeptide receptors for depression. Indeed, interest and research activity on neuropeptide receptors seems to have recently decreased. As a result, the clinical developments of NK2 antagonists saredutant (phase III) and SR-48,968 and the V1b antagonist nelivaptan SSR-149415 have been terminated.

However, given that most neuropeptides are synthesized in restricted brain regions and may have a distinct role depending on the course of stress exposure, patients who may respond to certain neuropeptide receptor agents should be selected based on their medical history and the severity of their illness. Therefore, although the outcomes of neuropeptide agents have been disappointing, their possible applications in specific subpopulations of depressed patients may be worth pursuing. Moreover, their efficacy against anxiety disorders has not been fully investigated in clinical studies, although some encouraging results have been obtained in clinical trials.

Nevertheless, numerous neuropeptide systems continue to be investigated, and proposed as potential therapeutic targets for depression and anxiety. In addition to the neuropeptide systems mentioned in this chapter, neuropeptide systems of interest in light of their involvement in depression and anxiety include neuropeptides related to the sleep-wake cycle, since depressive symptoms include abnormalities in sleep and circadian rhythms, and a decreased REM latency is highly replicable in depression.<sup>134,135</sup> For example, neuropeptide S and orexin, both of which increase arousal, have been reportedly involved in anxiety.<sup>136</sup> In particular, the involvement of orexin in panic disorder is of great interest, because it has been reported that CSF orexin levels are higher in human subjects with panic anxiety than in subjects without panic anxiety and orexin-1 antagonists attenuated panic responses (reduction of social interaction and increase in heart rate and blood pressure) in a rodent panic model.<sup>137</sup>

Therefore, neuropeptide receptors remain attractive targets for the development of treatments for depression and anxiety disorders, and clinical evaluations may eventually provide a clearer idea of the utility of neuropeptide receptor antagonists.

## References

1. M. S. Kramer, N. Cutler, J. Feighner, R. Shrivastava, J. Carman, J. J. Sramek, S. A. Reines, G. Liu, D. Snively, E. Wyatt-Knowles, J. J. Hale, S. G. Mills, M. MacCoss, C. J. Swain, T. Harrison, R. G. Hill, F. Hefti, E. M. Scolnick, M. A. Cascieri, G. G. Chicchi, S. Sadowski, A. R. Williams, L. Hewson, D. Smith, E. J. Carlson, R. J. Hargreaves and N. M. Rupniak, *Science*, 1998, **281**, 1640.

2. B. Binneman, D. Feltner, S. Kolluri, Y. Shi, R. Qiu and T. Stiger, *Am. J. Psychiatry*, 2008, **165**, 617.
3. G. Aguilera and C. Rabadan-Diehl, *Regul. Pept.*, 2000, **96**, 23.
4. W. Vale, J. Spiess, C. Rivier and J. Rivier, *Science*, 1981, **213**, 1394.
5. M. J. Owens and C. B. Nemeroff, *Pharmacol. Rev.*, 1991, **43**, 425.
6. C. B. Nemeroff, E. Widerlöv, G. Bissette, H. Walléus, I. Karlsson, K. Eklund, C. D. Kilts, P. T. Loosen and W. Vale, *Science*, 1984, **226**, 1342.
7. F. Holsboer, U. V. Bardeleben, A. Gerken, G. K. Stalla and O. A. Müller, *N. Engl. J. Med.*, 1984, **311**, 1127.
8. P. W. Gold, G. Chrousos, C. Kellner, R. Post, A. Roy, P. Augerinos, H. Schulte, E. Oldfield and D. L. Loriaux, *Am. J. Psychiatry*, 1984, **141**, 619.
9. G. Bissette, V. Klimek, J. Pan, C. Stockmeier and G. Ordway, *Neuropsychopharmacology*, 2003, **28**, 1328.
10. F. C. Raadsheer, W. J. Hoogendijk, F. C. Stam, F. J. Tilders and D. F. Swaab, *Neuroendocrinology*, 1994, **60**, 436.
11. Z. Merali, L. Du, P. Hrdina, M. Palkovits, G. Faludi, M. O. Poulter and H. Anisman, *J. Neurosci.*, 2004, **24**, 1478.
12. M. Ising, S. Horstmann, S. Kloiber, S. Lucae, E. B. Binder, N. Kern, H. E. Künzel, A. Pfennig, M. Uhr and F. Holsboer, *Biol. Psychiatry*, 2007, **62**, 47.
13. E. P. Zorrilla and G. F. Koob, *Drug Discov. Today*, 2010, **15**, 371.
14. S. Chaki, A. Nakazato, L. Kennis, M. Nakamura, C. Mackie, M. Sugiura, P. Vinken, D. Ashton, X. Langlois and T. Steckler, *Eur. J. Pharmacol.*, 2004, **485**, 145.
15. A. Surget, M. Saxe, S. Leman, Y. Ibarguen-Vargas, S. Chalon, G. Griebel, R. Hen and C. Belzung, *Biol. Psychiatry*, 2008, **64**, 293.
16. A. W. Zobel, T. Nickel, H. E. Künzel, N. Ackl, A. Sonntag, M. Ising and F. Holsboer, *J. Psychiatr. Res.*, 2000, **34**, 171.
17. M. Ising, U. S. Zimmermann, H. E. Künzel, M. Uhr, A. C. Foster, S. M. Learned-Coughlin, F. Holsboer and D. E. Grigoriadis, *Neuropsychopharmacology*, 2007, **32**, 1941.
18. J. E. Bailey, A. Papadopoulos, A. Diaper, S. Phillips, M. E. Schmidt, P. Ark, C. T. Dourish, G. R. Dawson and D. J. Nutt, *J. Psychopharmacol.*, 2011, **25**, 1199.
19. M. E. Schmidt, R. D. Andrews, P. V. D. Ark, T. Brown, E. Mannaert, T. Steckler, J. D. Hoon and K. V. Laere, *Psychopharmacology (Berl.)*, 2010, **208**, 109.
20. V. Coric, H. H. Feldman, D. A. Oren, A. Shekhar, J. Pultz, R. C. Dockens, X. Wu, K. A. Gentile, S. P. Huang, E. Emison, T. Delmonte, B. B. D'souza, D. L. Zimbroff, J. A. Grebb, A. W. Goddard and E. G. Stock, *Depress. Anxiety*, 2010, **27**, 417.
21. Y. L. Chen, R. S. Mansbach, S. M. Winter, E. Brooks, J. Collins, M. L. Corman, A. R. Dunaikis, W. S. Faraci, R. J. Gallaschun, A. Schmidt and D. W. Schulz, *J. Med. Chem.*, 1997, **40**, 1749.
22. C. Chen, K. M. Wilcoxon, C. Q. Huang, Y. Xie, J. R. McCarthy, T. R. Webb, Y. Zhu, J. Saunders, X. Liu, T. Chen, H. Bozigian and D. E. Grigoriadis, *J. Med. Chem.*, 2004, **47**, 4787.

23. R. S. Gross, Z. Guo, B. Dyck, T. Coon, C. Q. Huang, R. F. Lowe, D. Marinkovic, M. Moorjani, J. Nelson, S. Zamani-Kord, D. E. Grigoriadis, S. R. J. Hoare, P. D. Crowe, J. H. Bu, M. Haddach, J. McCarthy, J. Saunders, R. Sullivan, T. Chen and J. P. Williams, *J. Med. Chem.*, 2005, **48**, 5780.
24. J. E. Tellev, M. Lanier, M. Moorjani, E. Lin, Z. Luo, D. H. Slee, X. Zhang, S. R. J. Hoare, D. E. Grigoriadis, Y. S. Denis, R. D. Fabio, E. D. Modugno, J. Saunders and J. P. Williams, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7259.
25. R. D. Fabio, Y. St-Denis, F. M. Sabbatini, D. Andreotti, R. Arban, G. Bernasconi, S. Braggio, F. E. Blaney, A. M. Capelli, E. Castiglioni, E. D. Modugno, D. Donati, E. Fazzolari, E. Ratti, A. Feriani, S. Contini, G. Gentile, D. Ghirlanda, S. Provera, C. Marchioro, K. L. Roberts, A. Mingardi, M. Mattioli, A. Nalin, F. Pavone, S. Spada, D. G. Trist and A. Worby, *J. Med. Chem.*, 2008, **51**, 7370.
26. Y. L. Chen, J. Braselton, J. Forman, R. J. Gallaschun, R. Mansbach, A. W. Schmidt, T. F. Seeger, J. S. Sprouse, F. D. Tingley, III, E. Winston and D. W. Schulz, *J. Med. Chem.*, 2008, **51**, 1377.
27. Y. L. Chen, R. S. Obach, J. Braselton, M. L. Corman, J. Forman, J. Freeman, R. J. Gallaschun, R. Mansbach, A. W. Schmidt, J. S. Sprouse, F. D. Tingley, III, E. Winston and D. W. Schulz, *J. Med. Chem.*, 2008, **51**, 1385.
28. P. J. Gilligan, T. Clarke, L. He, S. Lelas, Y. Li, K. Heman, L. Fitzgerald, K. Miller, G. Zhang, A. Marshall, C. Krause, J. F. McElroy, K. Ward, K. Zeller, H. Wong, S. Bai, J. Saye, S. Grossman, R. Zaczek, S. P. Arneric, P. Hartig, D. Robertson and G. Trainor, *J. Med. Chem.*, 2009, **52**, 3084.
29. J. Kehne and S. D. Lombaert, *Curr. Drug Targets: CNS Neurol. Disord*, 2002, **1**, 467.
30. D. Gully, M. Geslin, L. Serva, E. Fontaine, P. Roger, C. Lair, V. Darre, C. Marcy, P. Rouby, J. Simiand, J. Guitard, G. Gout, R. Steinberg, D. Rodier, G. Griebel, P. Soubrie, M. Pascal, R. Pruss, B. Scatton, J. Maffrand and G. L. Fur, *J. Pharmacol. Exp. Ther.*, 2002, **301**, 322.
31. G. Griebel, J. Simiand, R. Steinberg, M. Jung, D. Gully, P. Roger, M. Geslin, B. Scatton, J. Maffrand and P. Soubri , *J. Pharmacol. Exp. Ther.*, 2002, **301**, 333.
32. R. F. D. Winter, A. M. V. Hemert, R. H. DeRijk, K. H. Zwinderman, A. C. Frankhuijzen-Sierevogel, V. M. Wiegant and J. G. Goekoop, *Neuropsychopharmacology*, 2003, **28**, 140.
33. L. V. Londen, J. G. Goekoop, G. M. V. Kempen, A. C. Frankhuijzen-Sierevogel, V. M. Wiegant, E. A. V. D. Velde and D. D. Wied, *Neuropsychopharmacology*, 1997, **17**, 284.
34. W. J. Inder, R. A. Donald, T. C. Prickett, C. M. Frampton, P. F. Sullivan, R. T. Mulder and P. R. Joyce, *Biol. Psychiatry*, 1997, **42**, 744.
35. J. S. Purba, W. J. Hoogendijk, M. A. Hofman and D. F. Swaab, *Arch. Gen. Psychiatry*, 1996, **53**, 137.
36. G. Meynen, U. A. Unmehopa, J. J. V. Heerikhuizen, M. A. Hofman, D. F. Swaab and W. J. Hoogendijk, *Biol. Psychiatry*, 2006, **60**, 892.

37. C. S. D. Kloet, E. Vermetten, E. Geuze, V. M. Wiegant and H. G. Westenberg, *J. Psychiatr. Res.*, 2008, **42**, 192.
38. M. Altemus, T. Pigott, K. T. Kalogeras, M. Demitrack, B. Dubbert, D. L. Murphy and P. W. Gold, *Arch. Gen. Psychiatry*, 1992, **49**, 9.
39. J. L. Abelson, J. L. Melledo and D. J. Bichet, *Neuropsychopharmacology*, 2001, **24**, 161.
40. E. R. Peskind, C. F. Jensen, M. Pascualy, D. Tsuang, D. Cowley, D. C. Martin, C. W. Wilkinson and M. A. Raskind, *Biol. Psychiatry*, 1998, **44**, 1007.
41. D. C. D. Goeij, D. Jezova and F. J. Tilders, *Brain Res.*, 1992, **577**, 165.
42. C. Rabadan-Diehl, S. J. Lolait and G. Aguilera, *J. Neuroendocrinol.*, 1995, **7**, 903.
43. G. C. Serradeil-Le, J. Wagnon, G. Valette, G. Garcia, M. Pascal, J. P. Maffrand and F. G. Le, *Prog. Brain Res.*, 2002, **139**, 197.
44. G. C. Serradeil-Le, J. Wagnon, J. Simiand, G. Griebel, C. Lacour, G. Guillon, C. Barberis, G. Brossard, P. Soubrié, D. Nisato, M. Pascal, R. Pruss, B. Scatton, J. P. Maffrand and G. L. Fur, *J. Pharmacol. Exp. Ther.*, 2002, **300**, 1122.
45. M. E. Breuer, M. M. V. Gaalen, W. Wernet, S. E. Claessens, R. S. Oosting, B. Behl, S. M. Korte, H. Schoemaker, G. Gross, B. Olivier and L. Groenink, *Naunyn Schmiedebergs Arch. Pharmacol.*, 2009, **379**, 101.
46. G. Griebel, J. Simiand, C. S. Gal, J. Wagnon, M. Pascal, B. Scatton, J. P. Maffrand and P. Soubrie, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 6370.
47. M. Iijima and S. Chaki, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 2007, **13**, 622.
48. N. Salome, J. Stemmelin, C. Cohen and G. Griebel, *Psychopharmacology (Berl.)*, 2006, **187**, 237.
49. J. Stemmelin, L. Lukovic, N. Salome and G. Griebel, *Neuropsychopharmacology*, 2005, **30**, 35.
50. T. Shimazaki, M. Iijima and S. Chaki, *Eur. J. Pharmacol.*, 2006, **543**, 63.
51. G. Griebel, J. Stemmelin, C. S. Gal and P. Soubrie, *Curr. Pharm. Des.*, 2005, **11**, 1549.
52. S. E. Napier, J. J. Letourneau, N. Ansari, D. S. Auld, J. Baker, S. Best, L. Campbell-Wan, J. Chan, M. Craighead, H. Desai, K. A. Goan, K. Ho, E. G. J. Hulskotte, C. P. MacSweeney, R. Milne, J. R. Morphy, I. Neagu, M. H. J. Ohlmeyer, A. W. M. M. Peeters, J. Presland, C. Riviello, G. S. F. Ruigt, F. J. Thomson, H. A. Zanetakos, J. Zhao and M. L. Webb, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1871.
53. C. S. Gal, J. Wagnon, J. Simiand, G. Griebel, C. Lacour, G. Guillon, C. Barberis, G. Brossard, P. Soubrié, D. Nisato, M. Pascal, R. Pruss, B. Scatton, J.-P. Maffrand and G. L. Fur, *J. Pharmacol. Exp. Ther.*, 2002, **300**, 1122.
54. M. Schönberger, C. Leggett, S. W. Kim and J. M. Hooker, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3103.
55. T. Oost, G. Backfisch, S. Bhowmik, M. Gaalen, H. Geneste, W. Hornberger, W. Lubisch, A. Netz, L. Unger and W. Wernet, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3828.

56. J. J. Letourneau, C. M. Riviello, H. Li, A. G. Cole, K. Ho, H. A. Zanetakos, H. Desai, J. Zhao, D. S. Auld, S. E. Napier, F. J. Thomson, K. A. Goan, J. R. Morphy, M. H. J. Ohlmeyer and M. L. Webb, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5394.
57. S. E. Napier, J. J. Letourneau, N. Ansari, D. S. Auld, J. Baker, S. Best, L. Campbell-Wan, R. Chan, M. Craighead, H. Desai, K. Ho, C. Mac Sweeney, R. Milne, J. R. Morphy, I. Neagu, M. H. J. Ohlmeyer, J. Pick, J. Presland, C. Riviello, H. A. Zanetakos, J. Zhao and M. L. Webb, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 3813.
58. T. Shimazaki, T. Yoshimizu and S. Chaki, *CNS Drugs*, 2006, **20**, 801.
59. D. Georgescu, R. M. Sears, J. D. Hommel, M. Barrot, C. A. Bolaños, D. J. Marsh, M. A. Bednarek, J. A. Bibb, E. Maratos-Flier, E. J. Nestler and R. J. DiLeone, *J. Neurosci.*, 2005, **25**, 2933.
60. D. G. Smith, E. T. Tzavara, J. Shaw, S. Luecke, M. Wade, R. Davis, C. Salhoff, G. G. Nomikos and D. R. Gehlert, *J. Neurosci.*, 2005, **25**, 914.
61. M. Roy, N. David, M. Cueva and M. Giorgetti, *Biol. Psychiatry*, 2007, **61**, 174.
62. P. Lagos, J. Urbanavicius, M. C. Scorza, R. Miraballes and P. Torterolo, *Behav. Brain Res.*, 2011, **218**, 259.
63. A. R. Kennedy, J. F. Todd, W. S. Dhillon, L. J. Seal, M. A. Ghatei, C. P. O'Toole, M. Jones, D. Witty, K. Winborne, G. Riley, G. Hervieu, S. Wilson and S. R. Bloom, *J. Neuroendocrinol.*, 2003, **15**, 268.
64. D. G. Smith, L. G. Hegde, T. D. Wolinsky, S. Miller, M. Papp, X. Ping, T. Edwards, C. P. Gerald and D. A. Craig, *Behav. Brain Res.*, 2009, **197**, 284.
65. B. Borowsky, M. M. Durkin, K. Ogozalek, M. R. Marzabadi, J. DeLeon, B. Lagu, R. Heurich, H. Lichtblau, Z. Shaposhnik, I. Daniewska, T. P. Blackburn, T. A. Branchek, C. Gerald, P. J. Vaysse and C. Forray, *Nat. Med.*, 2002, **8**, 825.
66. S. Chaki, T. Funakoshi, S. Hirota-Okuno, M. Nishiguchi, T. Shimazaki, M. Iijima, A. J. Grottick, K. Kanuma, K. Omodera, Y. Sekiguchi, S. Okuyama, T. A. Tran, G. Semple and W. Thomsen, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 831.
67. D. J. David, K. C. Klemenhagen, K. A. Holick, M. D. Saxe, I. Mendez, L. Santarelli, D. A. Craig, H. Zhong, C. J. Swanson, L. G. Hegde, X. I. Ping, D. Dong, M. R. Marzabadi, C. P. Gerald and R. Hen, *J. Pharmacol. Exp. Ther.*, 2007, **321**, 237.
68. S. Takekawa, A. Asami, Y. Ishihara, Y. Ishihara, J. Terauchi, K. Kato, Y. Shimomura, M. Mori, H. Murakoshi, K. Kato, N. Suzuki, O. Nishimura and M. Fujino, *Eur. J. Pharmacol.*, 2002, **438**, 129.
69. J. L. Méndez-Andino and J. A. Wos, *Drug Discovery Today*, 2007, **12**, 972.
70. D. R. Witty, J. H. Bateson, G. J. Hervieu, P. Jeffrey, C. N. Johnson, A. I. Muir, P. J. O'Hanlon, G. Stemp, A. J. Stevens, K. M. Thewlis, S. Wilson and K. Y. Winborn, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4865.
71. D. R. Witty, J. Bateson, G. J. Hervieu, K. Al-Barazanj, P. Jeffrey, D. Hamprecht, A. Haynes, C. N. Johnson, A. I. Muir, P. J. O'Hanlon, G.



- Stemp, A. J. Stevens, K. Thewlis and K. Y. Winborn, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4872.
72. D. L. Hertzog, K. A. Al-Barazanji, E. C. Bigham, M. J. Bishop, C. S. Britt, D. L. Carlton, J. P. Cooper, A. J. Daniels, D. M. Garrido, A. S. Goetz, M. K. Grizzle, Y. C. Guo, A. L. Handlon, D. M. Ignar, R. O. Morgan, A. J. Peat, F. X. Tavares and H. Zhou, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 4723.
73. M. D. Surman, E. E. Freeman, J. F. Grabowski, M. Hadden, A. J. Henderson, G. Jiang, X. Jiang, M. Luche, Y. Khmelnsky, S. Vickers, J. Viggers, S. Cheetham and P. R. Guzzo, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7015.
74. M. Hadden, D. M. Deering, A. J. Henderson, M. D. Surman, M. Luche, Y. Khmelnsky, S. Vickers, J. Viggers, S. Cheetham and P. R. Guzzo, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7020.
75. A. J. Henderson, D. Deering, J. F. Grabowski, M. Hadden, X. Jiang, Y. Khmelnsky, M. Luche, M. D. Surman, S. Cheetham, S. Vickers, J. Viggers and P. R. Guzzo, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7024.
76. C. Schwarzer, *Pharmacol. Ther.*, 2009, **123**, 353.
77. A. Pfeiffer, V. Brantl, A. Herz and H. M. Emrich, *Science*, 1986, **233**, 774.
78. W. A. Carlezon, Jr, C. Béguin, A. T. Knoll and B. M. Cohen, *Pharmacol. Ther.*, 2009, **123**, 334.
79. D. González, J. Riba, J. C. Bouso, G. Gómez-Jarabo and M. J. Barbanj, *Drug Alcohol Depend.*, 2006, **85**, 157.
80. G. V. Carr and I. Lucki, *Psychopharmacology (Berl.)*, 2010, **210**, 295.
81. S. D. Mague, A. M. Pliakas, M. S. Todtenkopf, H. C. Tomasiewicz, Y. Zhang, W. C. J. Stevens, R. M. Jones, P. S. Portoghesi and W. A. J. Carlezon, *J. Pharmacol. Exp. Ther.*, 2003, **305**, 323.
82. J. P. McLaughlin, S. Li, J. Valdez, T. A. Chavkin and C. Chavkin, *Neuropsychopharmacology*, 2006, **31**, 1241.
83. S. Grimwood, Y. Lu, A. W. Schmidt, M. A. Vanase-Frawley, A. Sawant-Basak, E. Miller, S. McLean, J. Freeman, S. Wong, J. P. McLaughlin and P. R. Verhoest, *J. Pharmacol. Exp. Ther.*, 2011, **339**, 555.
84. Y. Shirayama, H. Ishida, M. Iwata, G. I. Hazama, R. Kawahara and R. S. Duman, *J. Neurochem.*, 2004, **90**, 1258.
85. R. Bals-Kubik, A. Ableitner, A. Herz and T. S. Shippenberg, *J. Pharmacol. Exp. Ther.*, 1993, **264**, 489.
86. E. J. Nestler, M. Barrot, R. J. DiLeone, A. J. Eisch, S. J. Gold and L. M. Monteggia, *Neuron*, 2002, **34**, 13.
87. R. Spanagel, A. Herz and T. S. Shippenberg, *Proc. Natl Acad. Sci. USA*, 1992, **89**, 2046.
88. E. Chartoff, A. Sawyer, A. Rachlin, D. Potter, A. Pliakas and W. A. Carlezon, *Neuropharmacology*, 2011, **62**, 167.
89. G. V. Carr, D. A. Bangasser, T. Bethea, M. Young, R. J. Valentino and I. Lucki, *Neuropsychopharmacology*, 2010, **35**, 752.
90. R. Schwyzler, *Ann. NY Acad. Sci.*, 1997, **247**, 3.

91. P. S. Portoghese, A. W. Lipkowski and A. E. Takemori, *J. Med. Chem.*, 1987, **30**, 238.
92. R. M. Jones, S. A. Hjorth, T. W. Schwartz and P. S. Portoghese, *J. Med. Chem.*, 1998, **41**, 4911.
93. J. B. Thomas, R. N. Atkinson, R. B. Rothman, S. E. Fix, S. W. Mascarella, N. A. Vinson, H. Xu, C. M. Dersch, Y. F. Lu, B. E. Cantrell, D. M. Zimmerman and F. I. Carroll, *J. Med. Chem.*, 2001, **44**, 2687.
94. P. R. Verhoest, A. Sawant-Basak, V. Parikh, M. Hayward, G. W. Kauffman, V. Paradis, S. McHardy, S. McLean, S. Grimwood, A. W. Schmidt, M. Vanase-Frawley, J. Freeman, J. VanDeusen, L. Cox, D. Wong and S. Liras, *J. Med. Chem.*, 2011, **54**, 5868.
95. T. A. Brugel, R. W. Smith, M. Balestra, C. Becker, T. Daniels, G. M. Koether, S. R. Throner, L. M. Panko, D. G. Brown, R. Liu, J. Gordon and M. F. Peters, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5405.
96. T. A. Brugel, R. W. Smith, M. Balestra, C. Becker, T. Daniels, T. N. Hoerter, G. M. Koether, S. R. Throner, L. M. Panko, J. J. Folmer, J. Cacciola, A. M. Hunter, R. Liu, P. D. Edwards, D. G. Brown, J. Gordon, N. C. Ledonne, M. Pietras, P. Schroeder, L. A. Sygowski, L. T. Hirata, A. Zacco and M. F. Peters, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5847.
97. H. Hashimoto, H. Onishi, S. Koide, T. Kai and S. Yamagami, *Neurosci. Lett.*, 1996, **216**, 57.
98. C. Nilsson, G. Karlsson, K. Blennow, M. Heilig and R. Ekman, *Peptides*, 1996, **17**, 359.
99. M. Heilig, O. Zachrisson, A. Thorsell, A. Ehnvall, S. Mottagui-Tabar, M. Sjögren, M. Asberg, R. Ekman, C. Wahlestedt and H. Agren, *J. Psychiatr. Res.*, 2004, **38**, 113.
100. M. B. Stein, R. L. Hauger, K. S. Dhalla, M. J. Chartier and G. J. Asmundson, *Psychiatry Res.*, 1996, **59**, 183.
101. P. S. Widdowson, G. A. Ordway and A. E. Halaris, *J. Neurochem.*, 1992, **59**, 73.
102. G. Nikisch, H. Agren, C. B. Eap, A. Czernik, P. Baumann and A. A. Mathe, *Int. J. Neuropsychopharmacol.*, 2005, **8**, 403.
103. A. A. Mathe, *JECI*, 1999, **15**, 60.
104. P. A. Jimenez-Vasquez, D. H. Overstreet and A. A. Mathe, *J. Psychiatr. Res.*, 2000, **34**, 405.
105. A. A. Mathe, P. A. Jimenez, E. Theodorsson and C. Stenfors, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 1998, **22**, 529.
106. V. Sergeev, S. Fetissov, A. A. Mathé, P. A. Jimenez, T. Bartfai, P. Mortas, L. Gaudet, J. L. Moreau and T. Hökfelt, *Psychopharmacology (Berl.)*, 2005, **178**, 115.
107. H. Ishida, Y. Shirayama, M. Iwata and R. Kawahara, *Brain Res.*, 2005, **1046**, 239.
108. H. Husum, J. D. Mikkelsen, S. Hogg, A. A. Mathe and A. Mork, *Neuropharmacology*, 2000, **39**, 1463.
109. K. T. Britton, S. Southerland, E. V. Uden, D. Kirby, J. Rivier and G. Koob, *Psychopharmacology (Berl.)*, 1997, **132**, 6.

110. A. Kask, L. Rago and J. Harro, *Eur. J. Pharmacol.*, 1996, **317**, R3.
111. H. Ishida, Y. Shirayama, M. Iwata, S. Katayama, A. Yamamoto, R. Kawahara and K. Nakagome, *Hippocampus*, 2007, **17**, 271.
112. F. Bacchi, A. A. Mathe, P. Jimenez, L. Stasi, R. Arban, P. Gerrard and L. Caberlotto, *Peptides*, 2006, **27**, 3202.
113. A. Tschenett, N. Singewald, M. Carli, C. Balducci, P. Salchner, A. Vezzani, H. Herzog and G. Sperk, *Eur. J. Neurosci.*, 2003, **18**, 143.
114. R. O. Tasan, S. Lin, A. Hetzenauer, N. Singewald, H. Herzog and G. Sperk, *Neuroscience*, 2009, **158**, 1717.
115. M. W. Walker, T. D. Wolinsky, V. Jubian, G. Chandrasena, H. Zhong, X. Huang, S. Miller, L. G. Hegde, D. A. Marsteller, M. R. Marzabadi, M. Papp, D. H. Overstreet, C. P. Gerald and D. A. Craig, *J. Pharmacol. Exp. Ther.*, 2009, **328**, 900.
116. M. Packiarajan, M. R. Marzabadi, M. Desai, Y. Lu, S. A. Noble, W. C. Wong, V. Jubian, G. Chandrasena, T. D. Wolinsky, H. Zhong, M. W. Walker, O. Wiborg and K. Andersen, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5436.
117. R. E. Campbell, J. M. French-Mullen, M. A. Cowley, M. S. Smith and K. L. Grove, *Neuroendocrinology*, 2001, **74**, 106.
118. H. Doods, W. Gaida, H. A. Wieland, H. Dollinger, G. Schnorrenberg, F. Esser, W. Engel, W. Eberlein and K. Rudolf, *Eur. J. Pharmacol.*, 1999, **384**, R3.
119. C. J. Andres, I. A. Zimanyi, M. S. Deshpande, L. G. Iben, K. Grant-Young, G. K. Mattson and W. Zhaia, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2883.
120. J. A. Jablonowski, W. Chai, X. Li, D. A. Rudolph, W. V. Murray, M. A. Youngman, S. L. Dax, D. Nepomuceno, P. Bonaventure, T. W. Lovenberg and N. I. Carruthers, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1239.
121. G. E. Lunniss, A. A. Barnes, N. Barton, M. Biagetti, F. Bianchi, S. M. Blowers, L. Caberlotto, A. Emmons, I. P. Holmes, D. Montanari, R. Norris, D. J. Walters and S. P. Watson, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 4022.
122. G. E. Lunniss, A. A. Barnes, N. Barton, M. Biagetti, F. Bianchi, S. M. Blowers, L. L. Caberlotto, A. Emmons, I. P. Holmes, D. Montanari, R. Norris, G. V. Puckey, D. J. Walters, S. P. Watson and J. Willis, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 7341.
123. N. Sato, Y. Ogino, S. Mashiko and M. Ando, *Expert Opin. Ther. Patents*, 2009, **19**, 1401.
124. R. A. Adan, A. W. Szklarczyk, J. Oosterom, J. H. Brakkee, W. A. Nijenhuis, W. M. Schaaper, R. H. Melen and W. H. Gispen, *Eur. J. Pharmacol.*, 1999, **378**, 249.
125. T. Shimazaki and S. Chaki, *Pharmacol. Biochem. Behav.*, 2005, **80**, 395.
126. X. Y. Lu, G. S. Barsh, H. Akil and S. J. Watson, *J. Neurosci.*, 2003, **23**, 7863.
127. S. Chaki, S. Hirota, T. Funakoshi, Y. Suzuki, S. Suetake, T. Okubo, T. Ishii, A. Nakazato and S. Okuyama, *J. Pharmacol. Exp. Ther.*, 2003, **304**, 818.

128. S. Chaki, Y. Oshida, S. Ogawa, T. Funakoshi, T. Shimazaki, T. Okubo, A. Nakazato and S. Okuyama, *Pharmacol. Biochem. Behav.*, 2005, **82**, 621.
129. X. Lu, L. Sharkey and T. Bartfai, *CNS Neurol. Disord. Drug Targets*, 2007, **6**, 183.
130. J. M. Weiss, R. W. Bonsall, M. K. Demetrikopoulos, M. S. Emery and C. H. West, *Ann. NY Acad. Sci.*, 1998, **863**, 364.
131. A. Holmes, Q. Li, E. A. Koenig, E. Gold, D. Stephenson, R. J. Yang, J. Dreiling, T. Sullivan and J. N. Crawley, *Psychopharmacology (Berl.)*, 2005, **178**, 276.
132. C. J. Swanson, T. P. Blackburn, X. Zhang, K. Zheng, Z. Q. Xu, T. Hökfelt, T. D. Wolinsky, M. J. Konkel, H. Chen, H. Zhong, M. W. Walker, D. A. Craig, C. P. Gerald and T. A. Branchek, *Proc. Natl Acad. Sci. USA*, 2005, **102**, 17489.
133. A. M. Barr, J. W. Kinney, M. N. Hill, X. Lu, S. Biro, J. Rebek and T. Bartfai, *Neurosci. Lett.*, 2006, **405**, 111.
134. R. M. Benca, W. H. Obermeyer, R. A. Thisted and J. C. Gillin, *Arch. Gen. Psychiatry*, 1992, **49**, 651.
135. O. Berton and E. J. Nestler, *Nat. Rev. Neurosci.*, 2006, **7**, 137.
136. Y. L. Xu, R. K. Reinscheid, S. Huitron-Resendiz, S. D. Clark, Z. Wang, S. H. Lin, F. A. Brucher, J. Zeng, N. K. Ly, S. J. Henriksen, L. D. Lecea and O. Civelli, *Neuron*, 2004, **43**, 487.
137. P. L. Johnson, W. Truitt, S. D. Fitz, P. E. Minick, A. Dietrich, S. Sanghani, L. Träskman-Bendz, A. W. Goddard, L. Brundin and A. Shekhar, *Nat. Med.*, 2010, **16**, 111.

## **Section 3**

### **Other Psychiatric Disorders**



## CHAPTER 13

# *Therapeutic Approaches to Bipolar Disorder*

MOGHIS U. AHMAD, SHOUKATH M. ALI, SAIFUDDIN SHEIKH, ATEEQ AHMAD AND IMRAN AHMAD\*

Jina Pharmaceuticals Inc., 28100 N. Ashley Circle, Suite 103, Libertyville, Illinois 60048, USA

\*Email: Imran@jinapharma.com

## 13.1 Introduction

Bipolar disorder (BPD) or manic-depressive illness (MDI) is one of the most common and persistent mental illnesses. BPD is characterized by periods of deep and prolonged depression that alternate with periods of elevated and/or irritable mood, which are referred to as mania. The elevated moods are clinically referred to as mania and, if milder, called hypomania. Therefore, BPD is a serious life-long struggle and challenge. Symptoms of BPD would include a person experiencing both manic and depressive episodes, either in sudden shifts or even both at once. BPD today refers to the cycling between low- and high-mood episodes. Between these highs and lows, patients usually experience periods of higher functionality and can lead to a productive life.

Bipolar disorder has been subdivided into bipolar I, bipolar II, cyclothymia and other types, based on the nature and severity of mood episodes experienced.

**Bipolar I** refers to individuals who experience manic episodes, though major depressive episodes are not a preclusion to this form of bipolar disorder.



Bipolar I is also referred to as classic manic-depression, which is characterized by distinct episodes of major depression contrasting with episodes of mania, which lead to severe impairment of function.

**Bipolar II** refers to people who suffer from hypomanic episodes, as well as one or more major depressive episodes. In comparison, Bipolar II is a milder disorder consisting of depression alternating with periods of hypomania.

Hypomania is a less severe form of mania that does not lead to major impairment of social or occupational function. Bipolar II disorders are hard to diagnose because the bipolar symptoms of this type are not extreme enough to be obvious signs of a disorder. Therefore, for Bipolar I and Bipolar II disorder the specific courses of disorder are further classified as chronic, rapid cycling, catatonic or melancholic.

**Cyclothymia** is characterized by moods that fluctuate between mild and moderate depressive symptoms and hypomania. Cyclothymia can be a stable diagnosis or a prodromal phase leading to bipolar I or II disorder.

**Bipolar NOS** Bipolar disorder NOS stands for Not Otherwise Specified. This term is used to indicate bipolar illness that does not quite seem to fit with the above classifications for bipolar I or II disorders. NOS may be hard to identify, and professionals will usually conduct extensive interviews with friends and family members before starting treatment.

Data from the United States on lifetime prevalence vary, but indicate a rate of around 1% for bipolar I, 0.5–1% for bipolar II or cyclothymia and 2–5% for sub-threshold cases meeting some, but not all, criteria. The onset of full symptoms generally occurs in late adolescence or young adulthood. Diagnosis is based on the person's self-reported experiences, as well as observed behaviour. Evidence suggests that many people with creative talents have suffered from some form of bipolar disorder.<sup>1</sup>

BPD is a complex genetic disorder; however, it is likely caused by multiple different common diseases, each contributing relatively low risk for the disorder on their own. It is difficult to find such disease genes without a very large sample size, roughly thousands of subjects. Environmental factors are also considered to influence the disorder. BPD is often treated with mood stabilizer medications or other psychiatric drugs. Psychotherapy also has a role, often when there has been some recovery of stability. In serious cases in which there is a risk of harm to oneself or others, involuntary commitment may be used; these cases generally involve severe manic episodes with dangerous behaviour or depressive episodes with suicidal ideation.

## 13.2 Causes of Bipolar Disorder

The causes of BPD vary between individuals. Studies on twins have indicated a substantial genetic contribution, as well as environmental influence. The concordance rates for bipolar I, in modern studies, have been consistently reported to be around 40% in monozygotic twins (same genes) compared to 0–10% in dizygotic twins.<sup>2</sup> Findings from these studies yielded exciting new insights for a

combination of bipolar I and II and cyclothymia, the concordance rates of 42% vs. 11% with a relatively lower ratio for bipolar II that likely reflects heterogeneity. There is overlap with unipolar depression and if this is also counted in the co-twin the concordance with bipolar disorder rises to 67% (MZ) and 19% (DZ).<sup>3</sup> The relatively low concordance between dizygotic twins brought up together suggests that shared family environmental effects are limited, although the ability to detect them has been limited by small sample sizes.<sup>4</sup>

### 13.2.1 Genetic

Genetic studies have suggested that many chromosomal regions and candidate genes relate to the development of bipolar disorder, but the results are not consistent and often not replicated.<sup>5a</sup> A collaborative analysis of genetic studies suggested an association of two particular genes, ANK3 (ankyrin G) and CACNA1C (alpha 1C subunit of the L-type voltage-gated calcium channel).<sup>5b</sup> ANK3 is an adhesion protein found at axon initial segments that regulates the assembly of voltage-gated sodium channels and both ANK3 and subunits of the calcium channel are down-regulated in mouse brain in response to lithium, indicating a possible therapeutic mechanism of action of one of the most effective treatments for bipolar disorder. A review seeking to identify the more consistent findings suggested several genes related to serotonin (SLC6A4 and TPH2), dopamine (DRD4 and SLC6A3), glutamate (DAOA and DTNBP1) and cell growth and/or maintenance pathways (NRG1, DISC1 and BDNF), although a high risk of false positives is reported in the published literature. It was also suggested that individual genes are likely to have only a small effect and to be involved in some aspect related to the disorder.<sup>6</sup>

### 13.2.2 Childhood Precursors

Early childhood use of stimulants is found in large numbers of bipolar patients and has been found to cause an earlier onset of bipolar disorder and a worse clinical course, independent of attention deficit hyperactivity disorder.<sup>7</sup> Long-term studies indicate that children who receive a diagnosis of bipolar disorder at a later age may show subtle early traits such as sub-threshold cyclical mood abnormalities and full major depressive episodes. There may be hypersensitivity and irritability.

### 13.2.3 Neural Processes

Certain abnormalities in the structure and function of brain circuits could underlie bipolar and other mood disorders. Researchers reported their findings on several thousand MRI scans; however, their studies continue to report conflicting findings and there remains considerable debate over the neuroscientific findings. Given the size of the meta-analysis, it was concluded that

the relatively small number of significant findings was perhaps surprising, and that there may be a genuinely limited structural change in bipolar disorder or perhaps heterogeneity has obscured other differences. Based on several years of studies it was noted that average associations found at the level of multiple studies may not exist for an individual.<sup>8</sup>

#### **13.2.4 Melatonin Activity**

The hypersensitivity of the melatonin receptors in the eye could be a reliable indicator of bipolar disorder, as it is not dependent on state (mood, time, *etc.*). Hypersensitivity to light is called a trait marker. In small studies, patients diagnosed as bipolar reliably showed a melatonin-receptor hypersensitivity to light during sleep, causing a rapid drop in sleep time melatonin levels compared to control.<sup>9</sup> In a separate study, drug-free, recovered bipolar patients exhibited no hypersensitivity to light.<sup>10</sup> This area of research is not fully understood to the extent to which melatonin alterations may be a cause or effect of bipolar disorder.

#### **13.2.5 Psychological Processes**

Findings from psychological studies of BPD suggest that the period leading up to mania is often characterized by depression and anxiety at first, with isolated subclinical symptoms of mania such as increased energy and racing thoughts. There is also some indication that once mania has begun to develop, social stressors, including criticism from significant others, can further contribute. Findings from studies indicate that individuals may hold certain beliefs about themselves, their internal states and their social world that may make them vulnerable during changing mood states in the face of relevant life events. Symptoms are often likely to be continuous with normal experience. Once hypomania has developed, the activation level and impulsivity increase. Less notice may be taken of negative social reactions or advice and a person may be caught up in their own thoughts. There is some suggestion that the mood variation in bipolar disorder may not be cyclical as is often assumed, nor completely random, but results from a complex interaction between internal and external variables unfolding over time.<sup>11</sup>

#### **13.2.6 Life Events and Experiences**

Environmental factors also play a significant role in the development and course of BPD, and individual psychosocial variables may interact with genetic dispositions.<sup>6</sup> Findings from prospective studies indicate that recent life events and interpersonal relationships contribute to the likelihood of onsets and recurrences of bipolar mood episodes, as they do for onsets and recurrences of unipolar depression.<sup>12</sup> Between a third and a half of adults diagnosed with BPD report abusive experiences in childhood, which is associated with earlier onset

and more co-occurring disorders. The reported stressful events in childhood are higher in those with an adult diagnosis of bipolar spectrum disorder compared to those without, particularly events stemming from a harsh environment rather than from the child's own behaviour.<sup>13</sup>

## **13.3 Signs and Symptoms**

Bipolar disorder is a condition in which people experience abnormally elevated manic or hypomanic conditions and abnormally depressed states for periods of time in a way that interferes with functioning. There is no blood test to confirm the disorder and not everyone's symptoms are the same. BPD can appear to be unipolar depression. Often diagnosing BPD becomes difficult for medical professionals. What distinguishes BPD from unipolar depression is that the affected person experiences states of mania and depression. Often bipolar is inconsistent among patients because some people feel depressed more often and experience little mania whereas others experience predominantly manic symptoms.

### **13.3.1 Depressive Episode**

Signs and symptoms of the depressive phase of bipolar disorder include persistent feelings of sadness, anxiety, guilt, anger, isolation or hopelessness, disturbances in sleep and appetite, fatigue and loss of interest in enjoyable activities, problems concentrating, loneliness, self-loathing, apathy or indifference, depersonalization, loss of interest in sexual activity, shyness or social anxiety, irritability, chronic pain with or without a known cause, lack of motivation and suicidal ideation. In severe cases, the individual may become psychotic, which is also known as severe bipolar disorder.

### **13.3.2 Manic Episode**

Mania is generally characterized by a distinct period of an elevated, expansive or irritable mood state. People experience an increase in energy and a decreased need for sleep, and are easily distracted. A person's speech may be pressured, with thoughts experienced as racing. Judgement may become impaired; sufferers may go on spending sprees or engage in behaviour that is quite abnormal for them. They may indulge in substance abuse, such as alcohol or other depressants or sleeping pills. Their behaviour may become aggressive, intolerant or intrusive. Sexual drive may increase. People may feel out of control. They feel chosen, or on a special mission. A person in a manic state can begin to experience psychosis or a break with reality, where thinking is affected with mood.<sup>14</sup> Many people in manic state feel anxiety and very irritable to the point of rage while others are euphoric and grandiose.

### 13.3.3 Hypomanic Episode

Hypomania is a mild to moderate level of mania and is characterized by optimism, pressure of speech and activity and a decreased need for sleep. Hypomania is slightly different and does not inhibit functioning as mania does. In fact, many people with hypomania are more productive than usual. These people generally have increased energy and tend to become more active than usual. They do not have delusions or hallucinations. In general, hypomania does not inhibit functioning like mania. However, hypomania can be difficult to diagnose because it may masquerade as mere happiness, though it carries the same risks as mania. Hypomania may feel good to the person who experiences it, and even when family and friends start to recognize the mood swings, the individual often deny them.<sup>15</sup>

### 13.3.4 Mixed Affective Episode

In a mixed state of bipolar disorder, symptoms of mania and clinical depression occur simultaneously, such as confusion, fatigue, anxiety, agitation, aggressiveness or belligerence, impulsiveness, insomnia, irritability, panic, paranoia, pressure speech, racing thoughts, restlessness, rage and morbid and suicidal ideation.<sup>16</sup>

## 13.4 Diagnosis

An initial assessment of BPD includes a physical examination by a physician. At present there are no biological tests that can confirm BPD; however, tests are carried out to exclude other medical illnesses such as hypo- or hyperthyroidism, metabolic disturbance, a systemic infection or chronic disease and syphilis or HIV infection, *etc.* The most widely used criteria for diagnosing BPD are from the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) and the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10). The latter criteria are typically used in Europe and other regions while the DSM criteria are used in the USA and other regions, as well as prevailing in research studies.

There are lists of criteria for diagnosis. The first criterion is based on self-reported experiences of an individual as well as abnormalities in behaviour reported by family members and friends, followed by secondary signs observed by psychiatrist, clinical psychologist or clinician in a clinical assessment.

There are several other mental disorders that may involve similar symptoms to BPD. These include schizophrenia,<sup>17</sup> schizoaffective disorder, drug intoxication, brief drug-induced psychosis, schizophreniform disorder and borderline personality disorder. Both borderline personality and bipolar disorder can involve mood swings. The term "mood swings" refers to the cyclic episodes of elevated and depressed mood that generally last weeks or months. The term "borderline personality disorder" refers to the marked liability and reactivity of

mood, known as emotional deregulation, due to response to external psychosocial and intra-psychic stressors. These may arise or subside suddenly and dramatically and last for seconds, minutes, hours or days. According to some reports, borderline personality disorder represents a sub-threshold form of mood disorder,<sup>18,19</sup> while others maintain the distinctness, though noting that they often co-exist.<sup>20,21</sup>

Diagnosis of BPD is officially characterized such that, technically, anyone with a history of hypomania and depression has BPD, whatever their current or future functioning and vulnerability may be. This has been described as an “ethical and methodological issue”, as it means no one can be considered as being recovered from BPD according to the official criteria. This is considered problematic given that brief hypomanic episodes are widespread among people in general, and not necessarily associated with dysfunction.<sup>11</sup>

The diagnosis of BPD can be complicated by co-existing psychiatric conditions such as obsessive-compulsive disorder, panic disorder, social phobia or attention-deficit/hyperactivity disorder. Diagnosis becomes complicated if someone is abusing a substance. A careful analysis of symptoms and episodes, and possible discussions with friends and family, is crucial to establishing a valid treatment plan.<sup>22</sup> The diagnosis of BPD in children is particularly challenging, and controversial. Those who show some bipolar symptoms tend to have a rapid-cycling or mixed-cycling pattern that may not meet DSM-IV criteria.<sup>23</sup> Similarly, treatment of BPD in the elderly becomes complicated by the presence of dementia or the side-effects of medications being taken for other conditions.

## **13.5 Treatment of Bipolar Disorder**

The primary means of BPD treatment, medication, is correspondingly physical in nature and functions by directly manipulating brain and body chemistry. Only talk therapies are not appropriate as primary means of treatment, although they can be helpful and even essential as adjunctive treatments. In general, two types of medications, antidepressants and mood stabilizers, are used. Antidepressants help the patients to come out of depressive states, while mood stabilizers help to keep a patient's mood even and centred. There are also a few medications that have anti-manic properties and these are used occasionally. Bipolar patients treated with antidepressants alone are at high risk of swinging into mania. Similarly, patients treated with mood stabilizers alone often end up spending more time in dangerous depressive states than is necessary. For doctors it is easy to diagnose patients appropriately if they see them during a period of mania, but generally this does not happen, as patients feel good during the period of mania. Doctors generally start giving patients a course of antidepressants, only to find that patients become manic or hypomanic as a result. In such cases, the doctor adjusts and adds bipolar medications so as to respond quickly and appropriately to the new, more complex bipolar symptoms picture.

### 13.5.1 Pharmacotherapy

Medication treatment of BPD is the cornerstone of all modern therapeutic approaches, especially among long-term treatments. Treatments include medication, supportive psychotherapy and occasionally electroconvulsive therapy (ECT). Research and clinical experience clearly supports the use of mood stabilizers in long-term prophylaxis or prevention of new episodes of bipolar disorder. The three most effective mood stabilizers are lithium (lithium carbonate), carbamazepine (Tegretol) and valproate (Depakote). These agents are equally effective as acute phase treatment for mania, although lithium is usually used as the “first line” of treatment because of its long track record, relative safety and inexpensiveness. All three mood stabilizers are reliably measured by simple blood tests, a significant advantage for treatment. When these mood stabilizers are used for maintenance therapy they have been shown to reduce the number and severity of subsequent episodes, as well as to improve mood stability between episodes.

Mood stabilizers are also effective for the treatment of acute bipolar depression. They are generally not fast acting, and take two weeks or even longer to be effective. Depending on the nature of the mood instability, mood stabilizers are frequently supplemented with antipsychotics or antidepressants.

Before initiating any mood stabilizer, a careful medical history concentrating on cardiac, liver, renal and thyroid and the central nervous system should be undertaken. Also, the past and present history of drug use should be reviewed including prescription drugs, over-the-counter preparations, illicit drugs, alcohol, caffeine and nicotine usage. Other areas also need to be reviewed, such as dietary habits, weight change, exercise and recreational habits and sexual habits. The use of mood stabilizers during pregnancy is a complex issue and requires careful collaboration between OB/GYN and psychiatrists.

The list of BPD medications is extensive due to the complex and variable nature of the disorder and the complexities of individual reactions to medications, and side-effects in some. In the following sections, we describe and discuss the major medications used for bipolar disorder.

### 13.5.2 Lithium (Lithium Carbonate)

Lithium is a metallic element of the same family as sodium. It is abundant on the Earth's surface, and is administered as a medication in the form of a salt, lithium carbonate. Those seeking a “natural” treatment cannot find a more elemental treatment in all of modern medicine. Lithium is the only drug proven to reduce suicide in bipolar patients. In many ways it remains the best and most effective mood stabilizer available.<sup>24</sup> The mechanism of how lithium works is not completely understood, but it is clear that lithium affects the activity of neurotransmitters, the brain's chemical messengers, and more specifically regulates the responsiveness of brain cells.

Lithium is usually prescribed in conjunction with other bipolar medications, because its effects are not instant, but rather take some time to build up. Several



days or even weeks may need to pass before lithium's full therapeutic effects are apparent. Due to this lag time, other drugs are frequently administered to handle the acute phases of bipolar disorder mood episodes. Lithium's most common use with regard to BPD treatment is as a prophylactic (preventive) agent.

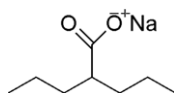
Normal side-effects of lithium include nausea, diarrhoea, increased urination, acne, thirstiness, muscle weakness, tremor, sedation and/or confusion. Most side-effects subside or dissipate over time and dosage adjustment often helps those that do not. Weight gain, hypothyroidism and increased urination are side-effects that are more common long-term concerns. Longer-term treatment may cause scarring of the kidneys, but this effect does not cause kidney failure. Medical practitioners generally obtain annual or semi-annual laboratory tests to monitor these changes. To avoid lithium toxicity, the patient must undergo blood monitoring to measure levels of lithium and make sure that they remain within an acceptable dose range. Overdose can be a serious medical emergency and toxic doses may lead to confusion, marked morbidity and even death. However, if taken as prescribed, lithium is quite safe. Most side-effects are manageable and the majority of people tolerate it well.

As is the case with most medication, care needs to be taken to avoid drug interactions when taking lithium or unanticipated and potentially negative side-effects may occur. Classes of medications that may interact with lithium include non-steroidal inflammatory drugs including ibuprofen (for example, Advil<sup>®</sup>), anti-hypertension drugs and antibiotics.

### 13.5.3 Valproate (Depakote, Depakene)

Valproic acid (also known as sodium valproate, Depakote or Depakene), is often prescribed as a stabilizing medication<sup>25</sup> for patients who do not tolerate lithium therapy. Sodium valproate takes a shorter period of time than lithium and therefore, it may be also used as a short-term bipolar disorder treatment when rapid mood stabilization is required. Sodium valproate (Figure 13.1) may be more effective than lithium for treating mania, rapid cycling bipolar disorder or mixed states. However, it is not as effective as lithium for the treatment of depressive states.

The side-effects of sodium valproate bipolar treatment are liver damage and decreased platelets; both the liver and platelets are important for maintaining normal blood functions. Other side-effects include nausea, sleepiness, increased appetite, weight gain, hair loss, decreased concentration and abdominal pain due to inflammation of the pancreas. Long-term use by women causes ovarian cysts that might possibly lead to infertility.



**Figure 13.1** Chemical structure of valproate.

Sodium valproate was initially discovered and used for the treatment of epilepsy, and classified as an anticonvulsant rather than a mood stabilizer. Therefore, patients taking other anticonvulsants should be aware that taking sodium valproate can cause their monitoring results to be skewed. Also, aspirin may cause increased levels of blood valproate. Combining any of these medications should be done with extreme caution and patients should inform their physicians if they are taking any other drugs.

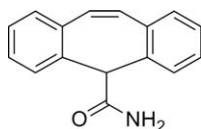
### 13.5.4 Carbamazepine (Tegretol)

Like sodium valproate, the anticonvulsant carbamazepine (Figure 13.2) was developed as an epilepsy treatment. Carbamazepine comes close to being an alternative first-line therapy to lithium. Although its usefulness to treat acute bipolar depression is still under investigation, carbamazepine is as effective as lithium for the treatment of mania, rapid mood cycling and mixed mood states. The mechanism of carbamazepine's anti-manic action is unknown, but it is believed to help stabilize the inner working of nerve cells, thus modulating brain signals.

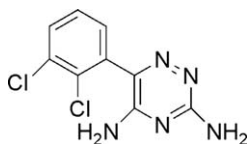
Because of its severe side-effects and potential for drug interaction, it no longer plays a significant role in BPD treatment. However, some patients respond better to carbamazepine than to other agents and, therefore, carbamazepine remains on the list of drugs used for BPD treatments. Although data are still lacking, carbamazepine appears to be as effective and safe as lithium for the treatment of bipolar disorder, in both acute and maintenance phases.<sup>26</sup> Carbamazepine and lithium may be used together when these medications have not been effective as single-drug strategies. Major side-effects of carbamazepine include nausea, vomiting, sleepiness or dizziness, confusion, decreased concentration, headache, double vision, abnormal heart rhythms and increased liver enzyme activity. Some less common side-effects of carbamazepine include potentially lethal forms of anaemia, decreased white blood cell production and a severe skin reaction known as the Stevens–Johnson Syndrome, requiring regular blood monitoring while the drug is taken.

### 13.5.5 Lamotrigine

Lamotrigine is the first anticonvulsant shown to be of benefit in bipolar depression.<sup>27</sup> Lamotrigine (Figure 13.3) behaves in a similar way to sodium valproate and carbamazepine when used as a BPD treatment. Lamotrigine has



**Figure 13.2** Chemical structure of carbamazepine.



**Figure 13.3** Chemical structure of lamotrigine.

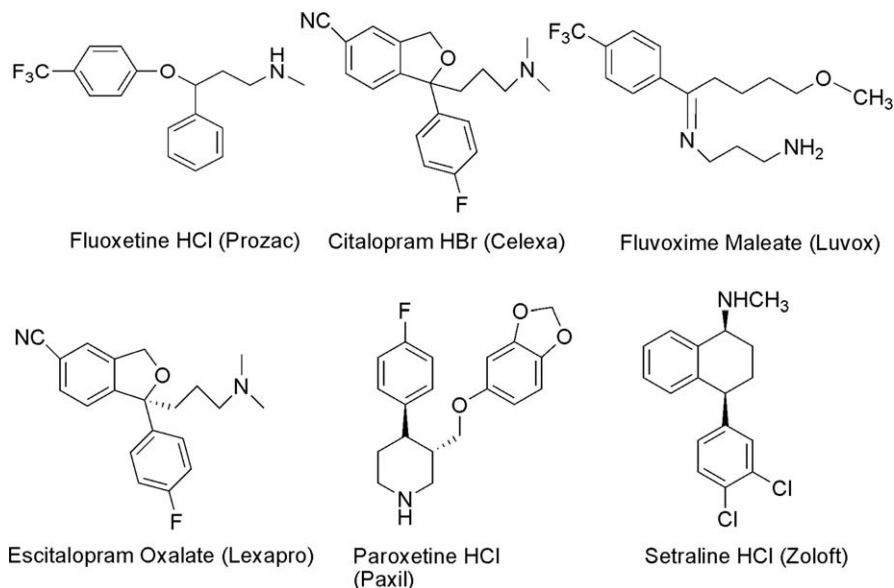
had been successful in controlling rapid cycling and mixed bipolar states in people who have not received adequate relief from carbamazepine and/or valproate. It also appears that lamotrigine has significantly more antidepressant potency than either carbamazepine or valproate. Unlike either valproate or carbamazepine, lamotrigine has an antidepressant effect as well an anti-manic effect.

Side-effects of lamotrigine include nausea, headache, insomnia, dizziness, sleepiness, blurred vision, double vision, lack of coordination and skin rashes. The side-effect of lamotrigine that most often causes the drug to be discontinued is a rash. Rashes can be mild, similar to slight sunburn, or can be quite severe, resembling a severe case of poison-ivy. A rash is more likely to develop when the initial doses of lamotrigine are high or when taking lamotrigine is started too rapidly when someone is taking valproate. Not everyone who develops rashes while taking lamotrigine goes on to discontinue the drug and someone with rashes can go on to have a good clinical response to continued therapy. Only a few interactions between lamotrigine and other drugs have been identified, *e.g.* lamotrigine increases the plasma level of carbamazepine and its metabolites. Carbamazepine lowers the concentration of lamotrigine in the blood. Valproate doubles the plasma level of lamotrigine, and the level of valproate decreased by about 25% in people taking lamotrigine. Interactions with other prescription and over-the-counter drugs are not known at this time.

### 13.5.6 Selective Serotonin Reuptake Inhibitors (SSRIs)

Antidepressants are used when acute bipolar depression does not respond to a mood stabilizer or a new episode of depression develops despite maintenance treatment. SSRIs are a newer class of antidepressant drugs. These medications work by preventing the neurotransmitter serotonin from being reabsorbed by the nerve cell that released it, thus forcing the serotonin to remain actively working and easing depression in patients. SSRIs are said to work by preventing the reuptake of serotonin (also known as 5-hydroxytryptamine, or 5-HT) by the pre-synaptic neuron, thus maintaining a higher level of 5-HT in the synapse. This class of drugs is given in Figure 13.4.

These antidepressants (Figure 13.4) typically have fewer adverse effects than tricyclic antidepressants (TCAs) or the monoamine oxidase inhibitors (MAOIs), and these effects are drowsiness, dry mouth, nervousness, anxiety, insomnia, decreased appetite, loose bowel movements, long-term weight gain



**Figure 13.4** Chemical structures of Selective Serotonin Reuptake Inhibitors.

and decreased ability to function sexually. These side-effects usually resolve within a few weeks of bipolar treatment. Recent research indicates that these drugs may interact with transcription factors<sup>28</sup> known as “clock genes”, which may play a role in the addictive properties of drugs (drug abuse) and possibly in obesity.<sup>29,30</sup>

Although SSRIs are effective treatments for depression, little is known about how SSRIs affect BPD patients. Despite this fact, SSRIs are a commonly prescribed treatment for bipolar disorder.

### 13.5.7 Serotonin-norepinephrine Reuptake Inhibitors (SNRI)

SNRIs are a newer form of antidepressant that works on norepinephrine, 5-HT and other neurotransmitter systems. These bipolar medications work similarly to SSRIs in that they inhibit the reuptake of neurotransmitters known to have an effect on mood at the synaptic junction. They typically have similar side-effects to the SSRIs, though there may be a withdrawal syndrome on discontinuation that may necessitate dosage tapering. The drugs in this category include Milnacipram (Ixel), Duloxetine (Cymbalta), Desvenlafaxine (Pristiq), Venlafaxine (Effexor) and Bupropion (Wellbutrin).

### 13.5.8 Noradrenergic and Specific Serotonergic Antidepressants (NaSSAs)

NaSSAs are a newer class of antidepressants, which increase norepinephrine (noradrenaline) and serotonin neurotransmission by blocking pre-synaptic

alpha-2 adrenergic receptors while at the same time blocking certain serotonin receptors. Side-effects include drowsiness, increased appetite and weight gain.<sup>31</sup> This class of drug includes Minserin (Tolvon) and Mirtazapine (Avanza, Zispin, Remeron).

### 13.5.9 Norepinephrine (Noradrenaline) Reuptake Inhibitors (NRIs)

NRIs act *via* norepinephrine (also known as noradrenaline). NRIs are considered to have positive effects on the concentration and motivation in particular. Examples of reuptake norepinephrine inhibitors include Viloxazine (Vivalan), Mazindol (Mazanor), Reboxetine (Edronax) and Atomoxetine (Stratters).

### 13.5.10 Tricyclic Antidepressants (TCAs)

TCAs are the oldest class of antidepressant drugs. These first-generation antidepressant medications also work by inhibiting reuptake of neurotransmitters. Tricyclics block the reuptake of certain neurotransmitters, such as norepinephrine (noradrenaline) and serotonin. This class of drug (Table 13.1) is not used very often due to the development of more selective and safer drugs. However, tricyclic antidepressants are still used in severe cases of major depression. Side-effects of tricyclic antidepressants include increased heart rate, drowsiness, dry mouth, constipation, urinary retention, blurred vision, dizziness, confusion and sexual dysfunction. These drugs are often lethal in overdoses, as they may cause a fatal arrhythmia.

### 13.5.11 Monoamine Oxidase Inhibitors (MAOIs)

MAOIs are used if other antidepressant medications are ineffective. Monoamine oxidase inhibitors work by blocking the enzyme monoamine oxidase, which breaks down the neurotransmitters serotonin, norepinephrine (noradrenaline) and dopamine. Because there are potentially fatal interactions between this class of medication and certain foods containing tyramine, as well as certain drugs, MAOIs are rarely prescribed nowadays. However, this does not apply to Emsam, the transdermal patch form of selegiline,<sup>32</sup> because it

**Table 13.1** List of Tricyclic Antidepressants (TCA's).

<i>Tertiary Amines</i>	<i>Secondary Amines</i>
Doxepin (Adapin)	Protriptyline (Vivactil)
Trimipramine (Surmontil)	Nortriptyline (Aventyl)
Amitriptyline (Elavil)	Aesipramine (Norpramin)
Clomipramine (Anafranil)	
Imipramine (Tofranil)	

bypasses the stomach and has a lesser possibility to induce such effects. MAOIs can be as effective as TCAs, although they are generally used less frequently due to the fact that they have a higher incidence of dangerous side-effects and interactions. A new class of MAOIs, moclobemide (Manerix), known as a Reversible Inhibitor of Monoamine oxidase A (RIMA), acts in a selective manner and does not require a special diet. The MAOI group of medicines includes selegiline (Eldepryl, Emsam), tranylcypromine (Parnate), phenelzine (Nardil), moclobemide (Aurorix, Manerix) and isocarboxazid (Marplan). Side-effects of MAOIs include insomnia, sleepiness, nervousness, low blood pressure, sweating, weight gain and sexual dysfunction. These bipolar medicines are considered as second-line therapies.

## 13.6 New Therapeutic Agents

The medications described above have proven helpful for many patients; however, there is a substantial group of those with BPD who have either not benefited from these options or experienced problematic side-effects. In the past two decades industry-sponsored clinical trials have brought few new treatments for bipolar depression, where our patients live most of their symptomatic lives.<sup>33</sup> There is a need to develop new life-long treatments for BPD that stabilize mood from below the baseline.<sup>34</sup> Researchers are committed to the development of new and improved treatments for BPD and have had to balance generalizability with the need to achieve assay sensitivity (separation from placebo) and, as a result, have made real but modest incremental progress.

With the exception of lithium, all of the current anti-manic agents are either anticonvulsant or antipsychotic drugs. It is interesting to note that no drug has been developed specifically for BPD. The lack of available therapeutics with a novel mechanism of action results in our modest understanding of the relevant molecular and cellular substrates of this complex emotional, behavioural and activity disorder. Drug development for mental disorders over the past five decades has been significantly stalled compared with other areas of medicine because different approaches are in order.<sup>35</sup> Different strategies have been proposed for the development of new and better drugs for patients with psychiatric disorders. We believe that we will be able to develop improved treatments for severe mood disorder. A growing number of studies and data suggest that mood disorders arise from abnormalities in synaptic and neuronal plasticity cascades, leading to aberrant information processing in critical synapses and circuits. Thus, these illnesses can best be conceptualized as genetically influenced disorders of synapses and circuits rather than simply as deficits or excesses in individual neurotransmitters. Recently, the US Food and Drug Administration approved the use of Saphris tablets for adults afflicted with BPD. This medication is commonly being used to treat bipolar I disorder and schizophrenia in adults. New research is being done into DNA and its connection with BPD. New methodologies are also being developed to look into DNA to identify variants of this disease.

### 13.6.1 Identification of Novel Molecular Target

The identification of biochemical targets of effective treatments has been facilitated by gene and protein expression profiling, which has led to the identification of several hitherto unexpected targets. Understanding of the molecular mechanisms presumably involved in mood stabilization has been gained through genome-wide gene expression profiling.<sup>36</sup> It is important to note that these methodologies are only a screening technique and the results need independent validation. The target validation for complex psychiatric disorders is so challenging that the following criteria for selecting mood stabilizers for further study were put forward: (i) corroboration of a target at the protein and functional level; (ii) observation with chemically different but clinically effective agents; (iii) occurrence at a dose/plasma level and time frame consistent with clinical therapeutic effect; (iv) localization to brain regions implicated in the neurobiology of the disorder under consideration; (v) when known, relevance to pathophysiology; and (vi) when possible, tethered to human genetic findings. Application of these stringent criteria led to protein kinase C (PKC) being recognized as a promising direct biochemical target for developing therapeutics to treat bipolar disorder.<sup>37,38</sup>

The mood stabilizers that meet the aforementioned criteria for further testing are Lithium (a monovalent cation) and valproate (an 8-carbon branched fatty acid). Lithium and valproate are structurally dissimilar, but researchers have postulated that both have similar therapeutic effects in the critical circuits of brain regions implicated in the pathophysiology of BPD. The therapeutic effects are specific to these agents and occur at drug concentrations *in vivo*. The resultant biochemical changes occur only after long-term administration with improvement of symptoms of the illness. Both lithium and valproate were found to bring the same effects on the PKC signalling cascade, actions that appear to be most pertinent to their anti-manic profile.

### 13.6.2 Protein Kinase C (PKC)

PKC is a family of structurally related isozyme subspecies with a heterogeneous distribution throughout the body.<sup>39,40</sup> There are at least 12 isoforms that differ in structure, subcellular localization, tissue specificity, mode of activation and substrate specificity.<sup>41</sup> The isoforms are subdivided into three classes (classical/conventional, novel and atypical) on the basis of activation requirements. Conventional PKC isoforms require calcium and diacylglycerol (DAG) for activation, whereas novel PKC isoforms that lack the C2 calcium-binding domain only require DAG for activation. Atypical PKC isoforms lack both C2 and DAG-binding C1 domains, and are not responsive to calcium or DAG, but respond to lipidic mediators such as phosphatidylinositol 3,4,5-triphosphate. Such isoforms are relevant to drug development, as directly targeting certain isoforms could bring about therapeutic effects (*e.g.* anti-manic). The targeting of isozymes in a discrete region rather than ubiquitously may minimize the adverse effects. The development of isozyme-specific compounds for



therapeutic use has demonstrated a major role in the management of several human disease conditions.

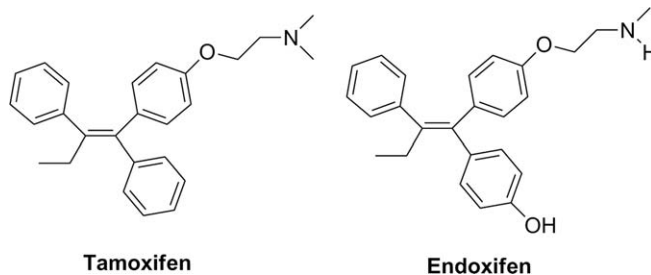
Activation of PKC results in its translocation, and subcellular localization is thought to regulate accessibility to activators and substrate. PKC is activated by varied upstream signals such as G protein-coupled receptors (GPDs), receptor tyrosine kinases (RTKs) and non-RTKs *via* diacylglycerol (DAG) activation. Several PKC isoforms are independently activated by the phospholipase C (PLC) and phosphoinositide-3 kinase (P13 K) pathways.<sup>41</sup>

### 13.6.3 PKC Signalling Cascade in Bipolar Disorder

The identification of the acute, *in vitro* effects of a number of anti-manic agents led investigators to develop criteria for relevant targets for future therapeutic agents.<sup>42,43</sup> The criteria as described above<sup>37,38</sup> recognize PKC as a promising direct biochemical target for developing therapeutics for BPD. A considerable amount of biochemical data supports the possible connection between PKC and the pathophysiology and treatment of BPD. Friedman *et al.*<sup>44</sup> studied PKC activity and PKC translocation in response to serotonin in platelets obtained from patients with BPD before and during lithium treatment. They found that the ratios of platelet membrane-bound to cytosolic PKC activities were increased in participants during a manic episode. In a *post mortem* study of brain tissue from patients with BPD, the same group of investigators<sup>45</sup> found increased PKC activity and translocation in the brains of patients with BPD compared with controls.

PKC signalling pathways are altered after treatment with lithium or valproate.<sup>37,42,44,46–48</sup> Long-term treatment with lithium in rats resulted in a significantly decreased PKC stimulation-induced release with phorbol esters in the cortex, hypothalamus and hippocampus; these areas have been implicated in mood disorder pathophysiology. PKC isozymes  $\alpha$  and  $\epsilon$  were reduced in the frontal cortex and hippocampus following lithium administration.<sup>42</sup> Similar to lithium, the other primary anti-manic drug, valproate, was also found to cause an isozyme-specific decrease in PKC  $\alpha$  and  $\epsilon$ .<sup>37</sup> Unlike lithium, the effects of valproate appear to be largely independent of myoinositol.<sup>42</sup> These two drugs act *via* different sub-pathways and this explains why patients who do not respond to lithium treatment may respond to valproate treatment or have some differences in efficacy based on manic syndrome profile (*e.g.* manic *vs.* mixed episode).

Stimulants, such as amphetamines, are capable of triggering manic episodes in susceptible individuals and induce manic-like behaviours in rodents,<sup>49</sup> and activate PKC and GAP-43 (growth-associated protein of 43 kDa) phosphorylation (implicated in neurotransmitter release).<sup>50–53</sup> The PKC inhibitor tamoxifen significantly reduced amphetamine-induced hyperactivity in a large open field without affecting spontaneous activity, and normalized amphetamine-induced increase to the centre of an open field, representing risk-taking behaviour; tamoxifen also attenuated amphetamine-induced phosphorylation of GAP-43.<sup>49</sup>



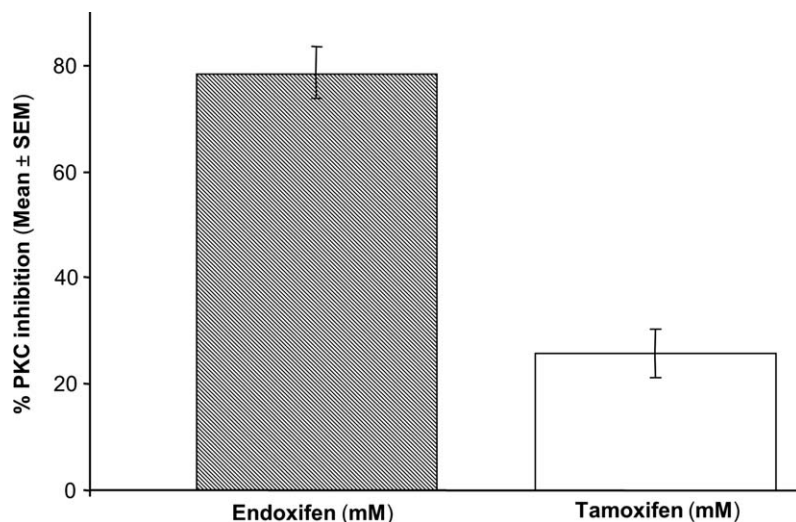
**Figure 13.5** Chemical structures of tamoxifen and endoxifen.

These data strongly suggested that further study of PKC inhibitors in humans with BPD was indicated. Tamoxifen (Figure 13.5) is only one relatively selective inhibitor of PKC available for human use that crosses the blood-brain barrier. Tamoxifen, a synthetic anti-oestrogen, is widely used in the treatment of breast cancer,<sup>54</sup> and is among the least toxic of the anticancer therapeutic agents. It has also been approved as a chemopreventive agent in women at high risk for breast cancer. Recently, it became apparent that tamoxifen possesses potent and selective PKC inhibitory effects at therapeutically relevant concentrations.<sup>55,56</sup> Furthermore, tamoxifen is brain penetrant, readily crossing the blood-brain barrier, and well tolerated even at doses 10-fold higher than routinely used in cancer treatment (up to 200 mg/day).<sup>57</sup>

There is a large inter-individual and ethnic variability in tamoxifen bioavailability and function due to CYP2D6 genetic polymorphism, which extensively metabolizes tamoxifen into active metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyl tamoxifen (endoxifen).<sup>58</sup> Recently, it was reported that alternative PKC inhibitors that are independent of CYP2D6 action such as endoxifen (Figure 13.5) may provide better opportunity for the treatment of bipolar disorder and other diseases (for example, oestrogen positive breast cancer).<sup>59</sup> Endoxifen inhibited PKC activity in a concentration-dependent manner. The percentage of PKC inhibition ranged between 12% and 78% with endoxifen concentration between 0.025 and 0.2 mM, respectively. In comparison, tamoxifen was found to be a less potent PKC inhibitor at 0.1 and 0.2 mM resulting in 14% and 25% PKC inhibition, respectively. Lower concentrations of tamoxifen (0.025 and 0.05 mM) showed negligible PKC inhibition. Percentage inhibition of PKC activity at 0.2 mM endoxifen and tamoxifen *in vitro* is shown in Figure 13.6. The result demonstrates that endoxifen is about four-fold more potent as a PKC inhibitor than tamoxifen, and strongly suggests its pivotal role in bipolar disorder. Endoxifen should be explored for proof-of-concept studies in comparison to tamoxifen in acute mania.

### 13.6.4 PKC inhibitors: Novel Therapeutics for Acute Mania?

As described above, the PKC signalling pathway appears to be a relevant and important target for the anti-manic actions of two structurally different



**Figure 13.6** Percentage inhibition of PKC activity by 0.2 M endoxifen and tamoxifen *in vitro*.

anti-manic agents (*i.e.* lithium and valproate). The PKC signalling pathway fulfils most of the criteria for therapeutic relevance. Thus, there is an obvious need to explore the potential efficacy of a direct PKC inhibitor in the treatment of acute mania. Based on the data generated in the last two decades, researchers embarked on proof-of-concept studies with tamoxifen in acute mania, and very recently endoxifen, which exhibited four-fold higher potency compared to tamoxifen.<sup>59</sup>

These studies confirm the hypothesis that directly inhibiting PKC improves manic symptoms. It is important to note that while these findings are encouraging, the results are preliminary and based on fairly small sample sizes. The results are to be confirmed in larger studies involving several hundred participants and with more selective PKC inhibitors. The evidence to date on multiple levels (*i.e.* preclinical and clinical studies) strongly supports further study of PKC inhibitors in bipolar disorder. Other selective PKC inhibitors are currently in different phases of clinical trial for the treatment of a variety of conditions (such as diabetic complications) and are possible candidates to test in bipolar disorder.<sup>60</sup> The issues regarding PKC isoform selectivity, brain penetrance and short- and long-term tolerability need to be thoroughly examined.

Regarding our current inability to predict who will respond to which medication and within what time frame, the evaluation of characteristics observed using valuable new technologies may provide a better understanding of the neurobiological basis involved in symptom improvement. It may also allow the identification of molecular targets for the next generation of treatments.

### 13.6.5 Omega-3 Fatty Acids in Bipolar Disorder

Omega-3 fatty acids may also be used as a treatment for BPD, particularly as a supplement to medication. These unique, natural fatty acids are used in several diseases such as heart disease, Crohn's disease, rheumatoid arthritis and cancer; they are also found to have mood-enhancing effects. Based on the finding the omega-3 fatty acids, given in the form of fish-oil tablets, have intra-cellular effects similar to those caused by lithium and sodium valproate. An initial clinical trial produced positive results.<sup>61</sup> However, this finding is not reproducible in several larger, double-blind clinical trials. It was hypothesized that the therapeutic ingredient in omega-3 fatty acid preparations is eicosapentaenoic acid (EPA) and that supplements should be high in this compound to be beneficial.<sup>62</sup> The interest was based mainly on the recognition that omega-3 fatty acids are endogenous, "natural" products with few side-effects and no known toxic effects.

Clinically, the study results provided early evidence of the efficacy of omega-3 fatty acids for adjunctive treatment of BPD.<sup>61</sup> If other studies confirm the efficacy, the omega-3 fatty acids can be considered to be "a nice mood stabilizer that has antidepressant properties and is nontoxic<sup>61</sup>". This type of mood therapy might be most useful for women during pregnancy or while breast feeding.

Biochemical studies of human white blood cells show that high-dose therapy with omega-3 fatty acids leads to the incorporation of these polyunsaturated fatty acids into the membrane phospholipids crucial for cell signalling.<sup>61</sup> Increased concentrations of omega-3 fatty acids in membrane phospholipids appear to suppress phosphatidylinositol-associated signal transduction pathways.<sup>63,64</sup> The mechanism of this effect is not clear. However, the incorporation of the omega-3 fatty acids into the lipid bilayer of the cell membrane alters the physical and chemical properties of the membrane, thus membrane phospholipids become more resistant to hydrolysis by phospholipases.<sup>65</sup> This could reduce the second messenger molecules, diacylglycerol and inositol triphosphate, and thereby produce less activation of downstream intra-cellular signalling molecules, such as protein kinase C (PKC) and calcium ions. It is possible that the omega-3 fatty acids also inhibit signal transduction mechanisms in the human central nervous system. Several studies<sup>66-69</sup> strongly suggest that the mechanism of action of mood stabilizers, such as lithium and valproate, involves a similar inhibition of post-synaptic signal transduction processes.

At present, more research is needed to clarify the role of omega-3 fatty acids in the treatment of bipolar disorder. If further studies confirm their efficacy in bipolar disorder, the omega-3 fatty acids may represent a new class of membrane-active psychotropic compounds and serve as a new class of rationally designed mood-stabilizing drugs.

## 13.7 Promising New Drugs under Clinical Development

Several therapeutic agents are currently under clinical development for patients having BPD symptoms. Some examples of drugs under development are

**Table 13.2** List of potential drugs under development.

Triacetyluridine (RG2417)	Lurasidone (SM-13496)
Pramipexole (Mirapex)	Staccato Loxapine (AZ-004)
Lu AA34893 (Lundbeck)	Nuvigil (armodafinil)
Lu AA39959 (Lundbeck)	Tamoxifen
RGH-188 (Cariprazine)	Endoxifen
Invega (Paliperidone)	

highlighted in Table 13.2. Pramipexole is a non-ergot dopamine agonist currently available for the treatment of the signs and symptoms of idiopathic Parkinson's disease. Pramipexole shows promise in treating cognitive deterioration related to bipolar depression. Preclinical studies of Lu AA39950 in animal models have shown antipsychotic-like as well as antidepressant-like effects, and are expected to show clear and convincing effects in patients with bipolar disorder. Drugs such as Invega, Lurasidone and Nuvigil are under clinical investigation for bipolar disorder. PKC inhibitors such as tamoxifen and endoxifen are potential drug candidates for the treatment of BPD. Endoxifen appears to be a superior candidate compared to tamoxifen for BPD treatment, especially for those with defects in activating enzymes for tamoxifen metabolism.

## 13.8 Other Treatment Options (Off-label Drugs) for Bipolar Disorder

Many clinicians have started experimental research with drugs that are indicated for the treatment of other illnesses, but have shown effective in the treatment of those with BPD. This type of medication usage is known as “off-label”. It is important to recognize that “off-label” drugs usage is an option only after all traditional treatment methods have failed. However, these drugs have not been approved by the Food and Drug Administration (FDA) for the treatment of BPD.

The “off-label” drugs used for the treatment of BPD belong to the group known as anticonvulsants, used primarily for the treatment of epilepsy. Several such drugs have shown promise in treating those with manic depression, particularly in helping stabilize mood. Some of the drugs are described below.

### 13.8.1 Neurontin (Gabapentin)

Neurontin is effective as a mood stabilizer for patients with BPD. It is chemically unrelated to any anticonvulsant. Neurontin appears to have fewer side-effects (*e.g.* weight gain, hair loss) than lithium and Depakote. Neurontin works better in alleviating mania than depression. It also seems to be a more potent anti-anxiety agent than both Depakote and carbamazepine (Tegretol).

Side-effects include sleepiness, dizziness, unsteadiness, nystagmus (rapid, involuntary fluctuation of the eyeballs), tremor and double vision.

### 13.8.2 Topamax (Topiramate)

Topamax has been the subject of a few open-label studies. The apparent advantage of this anticonvulsant over others is that it does not seem to cause weight gain. It actually helps in losing weight. It causes more cognitive side-effects than the other new drugs. The most common side-effects include sleepiness, dizziness, vision problems, unsteadiness, nervousness, nausea, speech problems, memory problems, tremor and confusion.

### 13.8.3 ABS-103

ABS-103 is in the early stages of clinical evaluation for its treatment potential for epilepsy, migraine headaches and mania. Evidence suggests it may be as effective as Depakote and it does not have many side-effects. In fact, ABS-103 might prove safe for women of childbearing age.

## 13.9 Conclusion

It is beyond the scope of this chapter to cover every aspect of bipolar disease and treatments available today. However, based on information available today, there is now strong evidence to support the view that targeting intracellular signalling cascades is a useful strategy in drug development for BPD. The findings suggest that PKC inhibition might be relevant to the anti-manic effects of lithium and valproate. Future studies examining more selective PKC inhibitors would need to take advantage of existing technologies (such as genetics) in an attempt to identify surrogate measures of outcome and to increase our understanding of the pathophysiology of the disease. We believe that the strategy of drug development research we have described in this chapter will, in due course, result in improved therapeutics for BPD.

In addition, the introduction of several new anti-epileptic drugs (AEDs) has demonstrated proven effectiveness in treating BPD. Most notable are Lamotrigine, demonstrating better efficacy with depressed bipolar patients, and Topiramate, with the generally desirable side-effects of weight loss and decreased appetite. The off-label use of medications described above has proven effective in certain studies; however, the medication is not approved by the FDA for this use. There is still a long way to go in getting approval by the FDA for their use in the treatment of bipolar disorder.

## References

1. D. Lam, K. Wright and N. Smith, *J. Affective Disord.*, 2004, **79**, 193.
2. T. Kieseppä, T. Partonen, J. Haukka and J. Lönqvist, *Am. J. Psychiatry*, 2004, **161**, 1814.



3. P. McGuffin, F. Rijdsdijk, M. Andrew, P. Sham and A. Cardno, *Arch. Gen. Psychiatry*, 2003, **60**, 497.
4. J. Edvardsen, S. Torgersen, E. Røysamb, S. Lygren, I. Skre, S. Onstad and P.A. Oien, *J. Affect Disord.*, 2008, **106**, 229.
5. a) T. Kato, *Psychiatry Clin. Neurosci.*, 2007, **61**, 3; b) M. Ferreira, M. O'Donovan, Y. A. Meng, I. D. Jones, M. Ruderferl and L. Jones, *Nat. Gen.*, 2008, **40**, 1056.
6. A. Serretti and L. Mandelli, *Mol. Psychiatry*, 2008, **13**, 742.
7. R. G. Ross, *Am. J. Psychiatry*, 2006, **163**, 1149.
8. M. J. Kempton, J. R. Geddes, U. Ettinger, S. C. R. Williams and P. M. G. Rasby, *Arch. Gen. Psychiatry*, 2008, **65**, 1017.
9. A. J. Lewy, J. I. Nurnberger and T. A. Wehr, *Am. J. Psychiatry*, 1985, **142**, 725.
10. L. J. Whalley, T. Perini, A. Shering and J. Bennie, *Psychiatry Res.*, 1991, **38**, 12.
11. W. Mansell and R. Pedley, *Clin. Psychol. Rev.*, 2008, **28**, 494.
12. L. B. Alloy, L. Y. Abramson, S. Urosevic, P. D. Walshaw, R. Nusslock and A. M. Neeren, *Clin. Psychol. Rev.*, 2005, **25**, 1043.
13. D. G. Louisa, B. A. Lauren and Y. A. Lyn, *J. Social Clin. Psychol.*, 2007, **26**, 460.
14. Bipolar Disorder: *NIH Publication No. 08-3679*, US National Institutes of Health (revised 2008).
15. Bipolar Disorder: *NIH Publication No.95-3679*, US National Institutes of Health (September 1995).
16. E. Goldman, *Clin. Psychiatr. News*, 1999, 25.
17. H. G. Pope, *Hospital and Community Psychiatry*, 1983, **34**, 322.
18. H. S. Akiskal, B. I. Yerevanian, G. C. Davis, D. Kinh and H. Lemmi, *Am. J. Psychiatry*, 1985, **142**, 192.
19. J. G. Gunderson and G. R. Elliott, *Am J. Psychiatry*, 1985, **142**, 277.
20. T. H. McGlashan, *Arch. Gen. Psychiatry*, 1983, **40**, 1319.
21. H. G. Pope, J. M. Jones, J. I. Hudson, B. M. Cohen and J. G. Gunderson, *Arch. Gen. Psychiatry*, 1983, **40**, 23.
22. D. Sagman and M. Tohen, *Psychiatric Times*, 2009, **26**(4), 1.
23. C. S. Kranowitz and R. Post, *Br. J. Psychiatry*, 1996, **168**, 314.
24. N. Poolsup, W. Po, A. Li and I. R. de Oliveira, *J. Clin. Pharm. Ther.*, 2000, **25**, 139.
25. K. Macritchie, J. R. Geddes, J. Scott, D. Haslam, M. S. de Lima and G. Goodwin, *The Cochrane Database of Systematic Reviews*, John Wiley and Sons, Ltd, 2002.
26. D. Ceron-Litvoc, B. G. Soares, J. Geddes, J. Litvoc and M. S. de Lima, *Hum. Psychopharmacol.*, 2009, **24**, 19.
27. J. R. Calabrese, C. L. Bowden, G. S. Sachs, J. A. Ascher, E. Monaghan and G. D. Rudd, *J. Clin. Psychiatry*, 1999, **60**, 79.
28. T. Uz, R. Ahmed, M. Akhisaroglu, M. Imbesi, A. A. Dirim and H. Manev, *J. Neurosci.*, 2005, **134**, 1309.
29. V. Yuferov, E. Butelman and M. Kreek, *Eur. J. Hum. Genet.*, 2005, **13**, 1101.



30. H. Manev and T. Uz, *Eur. J. Hum. Genet.*, 2006, **14**, 5.
31. G. L. Stimmel, J. A. Dopheide and S. M. Stahl, *Pharmacotherapy*, 1997, **17**, 10.
32. E. F. Cascade and A. H. Kalali, *Psychiatry*, June 2007.
33. L. L. Judd, H. S. Akiskal, P. J. Schettler, J. Endicott, D. A. Solomon, A. C. Leon, J. A. Rice and M. B. Keller, *Arch. Gen. Psychiatry*, 2002, **9**, 530.
34. T. A. Ketter and J. R. Calabrese, *J. Clin. Psychiatry*, 2002, **63**, 146.
35. T. R. Insel and E. M. Scolnick, *Mol. Psychiatry*, 2006, **11**, 11.
36. R. Zhou, C. A. Zarate and H. K. Manji, *Int. J. Neuropsychopharmacol.*, 2006, **9**, 263.
37. G. Chen, H. K. Manji, D. B. Hawver, C. B. Wright and W. Z. Potter, *J. Neurochem.*, 1994, **63**, 2361.
38. R. S. Jope, *Mol. Psychiatry*, 1999, **4**, 117.
39. G. Casabona, *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 1997, **21**, 407.
40. C. Tanaka and Y. Nishizuka, *Annu. Rev. Neurosci.*, 1994, **17**, 551.
41. M. Serova, A. Ghoul, K. A. Benhadji, E. Cvitkovic, S. Faivre, F. Calvo, F. Lokiec and E. Raymond, *Semin. Oncol.*, 2006, **33**, 466.
42. H. K. Manji and R. H. Lenox, *Biol. Psychiatry*, 1999, **46**, 1328.
43. J. T. Coyle and R. S. Duman, *Neuron*, 2003, **38**, 157.
44. E. Friedman, H. Y. Wang, D. Levinson, T. A. Connell and H. Singh, *Biol. Psychiatry*, 1993, **33**, 520.
45. H. Y. Wang and E. Friedman, *Biol. Psychiatry*, 1996, **40**, 568.
46. H. K. Manji, R. Etcheberrigaray, G. Chen and J. L. Olds, *J. Neurochem.*, 1993, **61**, 2303.
47. C. G. Hahn and E. Fredman, *Bipolar Disord.*, 1999, **1**, 81.
48. R. S. Seelan, A. Khalyfa, J. Lakshmanan, M. F. Casanova and R. N. Parthasarathy, *Neuroscience*, 2008, **151**, 1184.
49. H. Einat, P. Yuan, S. T. Szabo, S. Dogra and H. K. Manji, *Neuropsychobiology*, 2007, **55**, 123.
50. C. T. Giambalvo, *Neuropharmacology*, 1992, **31**, 1211.
51. M. E. Gnegy, P. Hong and S. T. Ferrell, *Mol. Brain Res.*, 1993, **20**, 289.
52. S. Iwata, G. H. Hewlett and M. E. Gnegy, *Synapse*, 1997a, **26**, 281.
53. S. Iwata, G. H. Hewlett, S. T. Ferrell, L. Kantor and M. E. Gnegy, *J. Pharmacol. Exp. Ther.*, 1997b, **283**, 1445.
54. V. C. Jordan, *Breast Cancer Res. Treat.*, 1994, **31**, 41.
55. K. Horgan, E. Cooke, M. B. Hallett and R. E. Mansel, *Biochem. Pharmacol.*, 1986, **35**, 4463.
56. W. T. Couldwell, M. H. Weiss, C. M. DeGiorgio, L. P. Weiner, D. R. Hinton, G. R. Ehresmann, P. S. Conti and M. L. J. Apuzzo, *Neurosurgery*, 1993, **32**, 485.
57. P. Tang, G. Roldan, P. M. Brasher, D. Fulton, W. Roa, A. Murtha, J. G. Cairncross and P. A. Forsyth, *J. Neurooncol.*, 2006, **78**, 311.
58. Information package, NOLVADEX<sup>®</sup> Product Monograph (Tamoxifen citrate), copyright AstraZeneca, Canada, Inc., 2000.
59. S. M. Ali, A. Ahmad, S. Shahabuddin, M. U. Ahmad, S. Sheikh and I. Ahmad, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 2665.

60. C. A. Zarate, Jr, J. Singh and H. K. Manji, *Biol. Psychiatry*, 2006, **59**, 1006–1020.
61. A. L. Stoll, W. E. Severus, M. P. Freeman, S. Rueter, H. A. Zboyan, E. Diamond, K. K. Cress and L. B. Marangell, *Arch. Gen Psychiatry*, 1999, **56**, 407.
62. Y. Osher, Y. Bersudsky and R. H. Belmaker, *J. Clin. Psychiatry*, 2005, **66**, 726.
63. L. Medini, S. Colli, C. Mosconi, E. Tremoli and C. Galli, *Biochem. Pharmacol.*, 1990, **39**, 129.
64. R. I. Sperling, A. I. Benincaso, C. T. Knoell, J. K. Larkin, K. F. Austen and D. R. Robinson, *J. Clin. Invest.*, 1993, **91**, 651.
65. P. G. Barton and F. D. Gunston, *J. Biol. Chem.*, 1975, **250**, 4470.
66. M. J. Berridge, C. P. Downes and M. R. Hanely, *Biochem. J.*, 1982, **206**, 587.
67. G. Chen, H. K. Manji, D. B. Hawver, C. B. Wright and W. Z. Potter, *J. Neurochem.*, 1994, **63**, 2361.
68. H. K. Manji, Y. Bersudsky, G. Chen, R. H. Belmaker and W. Z. Potter, *Neuropsychopharmacology*, 1996, **15**, 370.
69. I. A. Stoll and W. E. Severus, *Harv. Rev. Psychiatry*, 1996, **4**, 77.

## CHAPTER 14

# *Pharmacotherapies for Drug Addiction*

C. D. GIPSON AND P. W. KALIVAS\*

Department of Neurosciences, Medical University of South Carolina,  
173 Ashley Avenue, 205 DDB, Charleston, SC 29425, USA

\*Email: kalivasp@musc.edu

## 14.1 Introduction

Drug dependence is a leading cause of negative health consequences and has enormous societal impact.<sup>1</sup> Pharmacotherapies have been developed to target both the rewarding effects of drugs of abuse in order to block the pleasurable effects of drugs and therefore decrease use, as well as the chronic adaptations following drug use to inhibit relapse. Specifically, pharmacotherapies that reduce activation of the mesolimbic dopamine (DA) system via the dopamine transporter (DAT), the vesicular monoamine transporter (VMAT) or through DA D<sub>3</sub> receptor antagonism may have promise in the treatment of substance abuse by reducing the rewarding effects of these drugs. In addition to direct action on the DA system, other targets have been identified in drug addiction that have shown promise in both reducing the rewarding effects of drugs of abuse, as well as inhibiting relapse of drug seeking behaviour. Targeting the opioid system has shown promise in decreasing the rewarding effects of drugs of abuse. For example, antagonism of the  $\mu$  opioid receptor has shown promise in reducing alcohol use both clinically and preclinically, as this receptor has been primarily implicated in drug reinforcement and dependence.

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

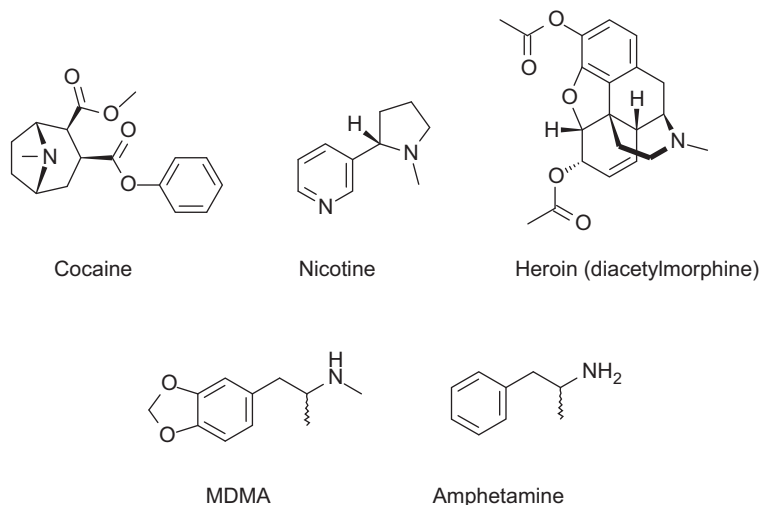
Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

Chronic use of addictive drugs has been found to produce enduring neuroadaptations in the corticostriatal brain circuitry involved in the plasticity of learning and behaviour. Enduring vulnerability to relapse is a primary component of drug addiction, and thus has been proposed to be a key point of pharmacotherapeutic intervention in treating addiction.<sup>2</sup> Indeed, impairment in glutamate homeostasis between glia and synapses has been proposed,<sup>3</sup> such that there is an impairment of synaptic plasticity and neuroadaptations in glutamatergic signalling in response to chronic drug use. Specifically, targeting the cystine-glutamate exchanger or glutamate transporters following withdrawal from chronic use of cocaine has shown promise in treating relapse to drug-seeking behaviour. Indeed, furthering our understanding of the neuroadaptations that occur during this critical point in the addiction cycle is imperative for developing effective pharmacological interventions for relapse.

In pharmacotherapeutic development, drugs are designed to bind to specific molecular targets, and this interaction between the drug and its target will either prompt or block biological processes that result in therapeutic effects. Often in drug development, there is not absolute selectivity for these targets and therefore these drugs will likely interact with other targets, specifically those with similar structure to the primary target. The effect of this is that most drugs possess multiple actions, and this can cause unwanted side-effects and will likely lead to issues with patient compliance. Thus, more work is needed to find more selective pharmacotherapies that have fewer side-effects to improve compliance among individuals with addictions. As well, individual treatment is needed to improve efficacy of pharmacotherapy. In this chapter, we will discuss the various targets identified in both the rewarding effects of drugs, as well as pharmacotherapies developed to reverse chronic neuroadaptations that occur in response to chronic drug use and thus reduce relapse to drug seeking.

## **14.2 Dopamine Transporter and D<sub>3</sub> Receptors: Implications for Pharmacotherapy**

Nearly all abused substances activate the mesolimbic DA pathway.<sup>4,5</sup> The neurotransmitter traditionally implicated in drug abuse is DA.<sup>6,7</sup> DA release within the prefrontal cortex (PFC)-nucleus accumbens (NA)-ventral pallidum (VP) series circuit strongly contributes to the acquisition of drug-taking and reinforces behaviours that support obtaining drug. Dopamine also contributes to relapse to drug-seeking behaviour in animal models of relapse.<sup>8</sup> The molecular binding site and physiological changes that promote dopamine transmission are distinct between different classes of abused drug. For example, amphetamine-like psychostimulants bind to DA transporters (DAT; the membrane-spanning protein that transports DA into the cytosol from the extra-cellular space). Cocaine binds to the transporter preventing DA removal from the synaptic cleft and thereby increases the extra-cellular accumulation of



**Figure 14.1** Common drugs of abuse.

action potential-mediated DA release. Most amphetamines (*e.g.* methamphetamine, MDMA, Figure 14.1) also act on DAT, but function as a false dopamine substrate causing the reverse transport of DA into the extra-cellular space. Some amphetamines also are transported into the DA synaptic vesicles. Sequestering amphetamines in the vesicle degrades the proton gradient needed for vesicular accumulation of DA, thereby causing DA to accumulate in the cytosol and promote reverse transport of DA into the extra-cellular space in exchange for amphetamine. Thus, unlike cocaine (Figure 14.1), amphetamine can cause release of dopamine in the absence of action potentials. Interestingly, alcohol dependence may also involve DAT in the mesolimbic DA reward pathway,<sup>9</sup> since epigenetic regulation of the DAT promoter via DNA methylation is increased during alcohol withdrawal in patients.<sup>10</sup> Due to its status as a primary target of various drugs of abuse, DAT is of great interest for pharmacotherapeutic targeting and intervention.<sup>11</sup>

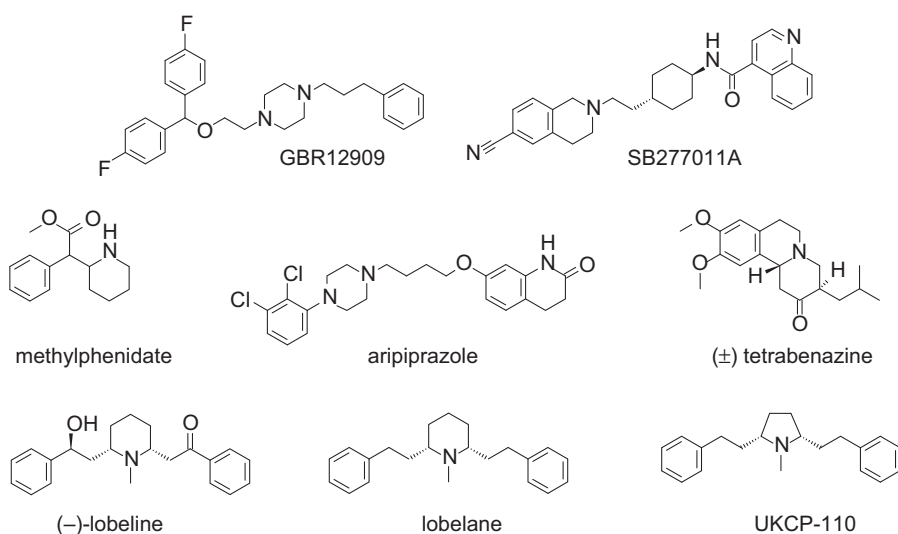
In addition to DAT as a pharmacotherapeutic target, evidence suggests a central role for DA D<sub>3</sub> receptors in drug addiction. D<sub>1</sub> and D<sub>2</sub> receptors have been widely implicated in drug addiction and, specifically, the reinforcing effects of cocaine;<sup>12</sup> however, side-effects associated with blockade of either receptor makes them poor targets for treating substance abuse disorders. In contrast, although members of the D<sub>2</sub> receptor family, D<sub>3</sub> receptors may have potential as effective pharmacotherapeutic targets for treating drug abuse with reduced side-effects.<sup>13</sup> Unlike the D<sub>1</sub> and D<sub>2</sub> receptor subtypes that are widely distributed in brain, the D<sub>3</sub> receptor has a relatively limited distribution, with highest density in regions of mesolimbic dopamine axon terminals, in particular the NA.<sup>14,15</sup> Previous research has shown that several dopamine D<sub>3</sub> agonists decrease cocaine self-administration in rats.<sup>16</sup> As well, selective D<sub>3</sub> antagonists

significantly reduce stress-, drug- and cue-induced reinstatement of drug seeking behaviour, a preclinical model of relapse.<sup>17</sup>

### 14.2.1 Alcohol

In contrast to cocaine or opioids, drugs for which their role in modulating dopamine neurotransmission has been better characterized, alcohol acts on a broader range of neurochemical systems than other addictive drugs.<sup>18</sup> Thus, therapeutic development for alcohol addiction remains difficult as any one of these systems can modulate neuroadaptations leading to alcohol addiction.<sup>19</sup> Several pharmacotherapies have been implemented in the treatment of alcohol abuse, including acamprosate, naltrexone and disulfiram (Antabuse). Although disulfiram has been shown to be efficacious in preventing the relapse of alcohol use among detoxified patients, compliance among non-detoxified alcoholics is quite low because they become ill if they drink alcohol while taking this medication. While compliance for naltrexone is better among alcoholics, improvement is necessary.

It has long been established that ethanol consumption stimulates DA release in the NA in rats.<sup>20</sup> Preclinically, blocking DAT with GBR-12909 (Figure 14.2) significantly decreases ethanol consumption in rats.<sup>21</sup> As well, DAT is markedly lower in alcoholics during early withdrawal, but increases following 4 weeks of withdrawal.<sup>22</sup> These results suggest that a decrease in DAT during prolonged heavy drinking may be an underlying risk factor in early relapse, and that a pharmacotherapy designed to rescue DAT levels during early



**Figure 14.2** Potential pharmacotherapies targeting dopaminergic signaling to reduce drug dependence.

withdrawal may be an effective treatment strategy for alcohol relapse. In addition to effects on DAT, prolonged alcohol use has been found to up-regulate  $D_3$  receptors, which may promote increased alcohol seeking and relapse.<sup>23</sup> Although DAergic mechanisms have been found to play an important role in alcohol consumption and withdrawal, pharmacotherapeutic targeting of this system in alcohol abuse is not well established. Preclinically, SB-277011A (a DA  $D_3$  receptor antagonist, Figure 14.2) produced a significant decrease in alcohol intake, preference and responses in a two-bottle choice paradigm of alcohol self-administration in alcohol-preferring rats, compared to their non-preferring counterparts.<sup>24</sup> As well, SB-277011A did not produce locomotor side-effects, indicating that antagonizing the  $D_3$  receptor in alcohol addiction may be a viable avenue of pharmacotherapeutic development in alcohol addiction.

### 14.2.2 Cocaine and Methamphetamine

To date, no effective treatments have been developed for cocaine and methamphetamine dependence. As a treatment possibility for cocaine, agonist replacement therapy with the DAT inhibitors having a favourable pharmacokinetic profile for limiting abuse liability (*e.g.* slow onset and long duration of action) have been proposed and evaluated preclinically. Methylphenidate (Figure 14.2) has been formulated for this purpose in clinical trials and shown to be effective in producing psychoactive side-effects similar to cocaine (*e.g.* ratings of having “more energy” and “eating less”, respectively) without reports of increases in craving.<sup>25</sup> As well, individuals addicted to cocaine have reported that intravenous methylphenidate is similar to cocaine. Methylphenidate occupies DAT but has slower clearance than cocaine,<sup>26</sup> and therapeutic doses of this drug occupy up to 50% of DAT as measured by positron emission tomography (PET).<sup>27</sup>

Several pharmacotherapies have failed to show efficacy in treating methamphetamine dependence, including the high-affinity DA  $D_2$  receptor partial agonist aripiprazole,<sup>28</sup> the GABAergic agent gabapentin (also found to be an  $\alpha 2\delta 1$  ligand and thus competes with thrombospondin for binding to this receptor<sup>29</sup>) and serotonergic agents such as ondansetron and mirtazepine<sup>30</sup> (Figure 14.2). Pharmacotherapies for methamphetamine have focused on blocking the vesicular monoamine transporter-2 (VMAT2) as well as DAT, as methamphetamine not only blocks DAT, but also blocks monoamine oxidase (MAO) and reverses VMAT2 transport. Specifically, analogues of *Lobelia inflata*, a plant with a long history of therapeutic use as a respiratory stimulant and in tobacco smoking cessation, act as potent antagonists at  $\alpha 3\beta 2$  and  $\alpha 4\beta 2$  neuronal nicotinic receptor subtypes,<sup>31</sup> and have been developed preclinically to inhibit methamphetamine self-administration via VMAT2 and DAT inhibition without decreasing food intake. For example, lobelane, an analogue of lobeline (Figure 14.2), dose-dependently decreased methamphetamine intake, although tolerance developed to this effect.<sup>32</sup> More recently,



*cis*-2,5-di-(2-phenethyl)-pyrrolidine hydrochloride (UKCP-110, Figure 14.2), a novel derivative of nor-lobelane, inhibited VMAT2 function, decreased methamphetamine-evoked endogenous DA release in striatal slices and decreased methamphetamine self-administration without decreasing food-reinforced behaviour in rats.<sup>33</sup> Although a small decrease in food intake is possibly preferable, one complication in targeting the DAergic system in drug discovery is the possibility of inhibiting food intake due to decreases in the reward pathway associated with natural rewards. Thus, it is important to examine the effects of these compounds on food intake preclinically prior to engaging a clinical level of analysis.

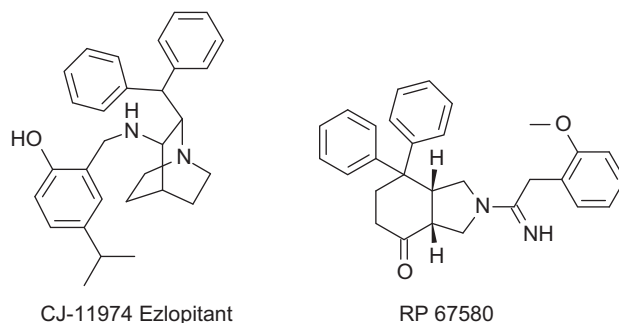
In addition to analogues of lobeline, tetrabenazine (Figure 14.2) provides another avenue of pharmacotherapeutic development for targeting VMAT2 in the treatment of methamphetamine dependence. Tetrabenazine is a derivative of benzoquinolizine that is approved in the treatment of dyskinesias in Europe, and binds with high affinity to VMAT2, inhibiting uptake of cytosolic monoamines. Tetrabenazine is currently being tested preclinically to determine if it has potential as a therapeutic treatment of methamphetamine dependence in the United States, as it decreases methamphetamine-induced locomotor activity and fully blocks the discriminative stimulus effects of methamphetamine and decreases methamphetamine self-administration in rats.<sup>34</sup> Further studies are underway at the NIDA Addiction Treatment Discovery Program (ATDP) to further evaluate tetrabenazine as an effective treatment of methamphetamine dependence.<sup>35</sup>

## 14.3 Substance P and the NK1 Receptor

Substance P is a member of the tachykinin family of neuropeptides, and is one of the most abundant neuropeptides in the brain that has been implicated in addiction and stress.<sup>36,37</sup> Interestingly, the neurokinin substance P and its NK1 receptor are highly expressed in key regions of the pathway frequently implicated in addiction, including the amygdala and NA<sup>38</sup> (see Figure 14.4). As well, NK1 receptor antagonists (Figure 14.3) have anxiolytic and antidepressant qualities, both clinically and preclinically, and they have been shown to have antitumour action and inhibit the migration of tumour cells.<sup>39–41</sup> The NK1 receptor and  $\mu$  opioid receptors colocalize in regions such as the locus coeruleus (LC) and NAc shell, key regions implicated in reward. Indeed, substance P signalling appears to be important in the behavioural responses to opioids, and thus important interactions may occur between these pathways in addiction. As well, neurons in key regions involved in addiction such as NA and VP may be involved in these interactions.<sup>36</sup>

### 14.3.1 Alcohol

New pharmacotherapeutic targets for alcohol addiction include substance P and the NK1 receptor, among others.<sup>42</sup> Preclinically, the NK1 receptor



**Figure 14.3** NK1 antagonists.

antagonist L822429 inhibits stress-induced reinstatement of alcohol-seeking behaviour in rats, suggesting that NK1 receptor antagonists may be an avenue of therapeutic development for inhibiting stress-induced craving and alcohol relapse.<sup>43</sup> Additionally, the NK1 receptor antagonist ezlopitant (CJ-11, 974) reduces appetitive responding for sucrose and ethanol in rodents without affecting locomotor activity or water intake, suggesting this target in the treatment of both obesity and alcohol dependence.<sup>44</sup>

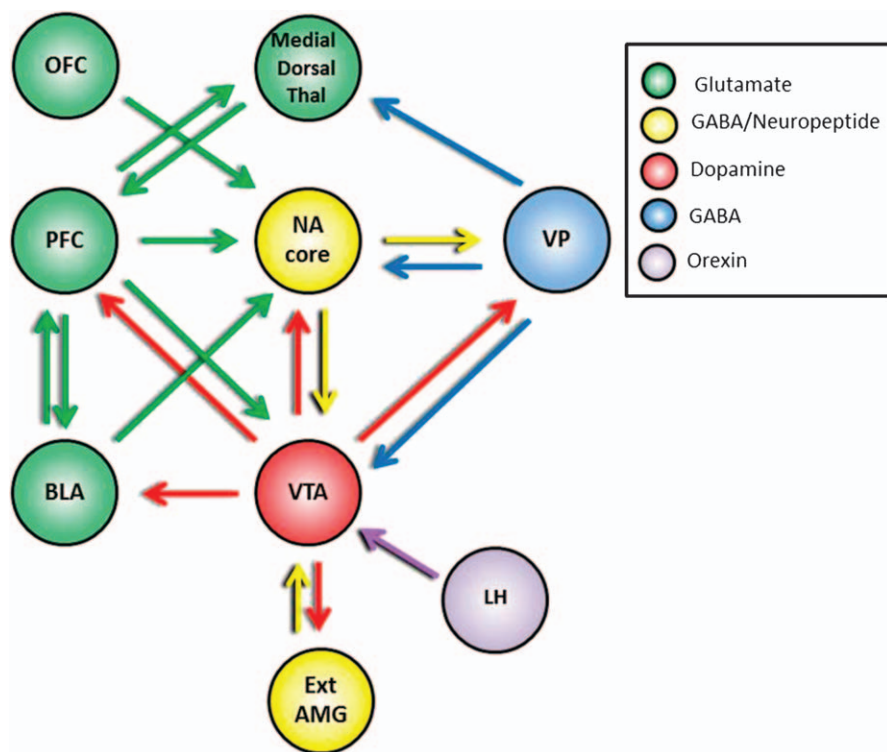
### 14.3.2 Opioid Addiction

Although a new avenue of research, the NK1 receptor has been implicated in the treatment of opioid addiction. Preclinically, NK1 receptor-lacking mice show a loss of morphine self-administration and behavioural sensitization, but not cocaine self-administration,<sup>45</sup> thus the NK1 receptor is essential for opioids to produce their rewarding effects in animals. As well, the NK1 receptor antagonist, RP 67580, decreases naloxone-precipitated morphine withdrawal symptoms in rats.<sup>46</sup> Even from the still early data on the role of the NK1 receptor in opioid addiction, we can surmise that this could be a productive avenue of therapeutic development.

## 14.4 Glutamatergic Signalling: Dysregulation in Addiction and Pharmacological Targeting

A portion of the brain circuit involved in relapse to the use of addictive substances, the projection from the prefrontal cortex (PFC) to the NA, undergoes pathological changes in protein content and extra-cellular glutamate levels that strongly contribute to relapse of drug-seeking behaviour in animal models. Indeed, there is extensive evidence for the role of the cortico-accumbens circuitry in drug relapse. For example, glutamatergic input from PFC to NA has been found to underlie heroin-, cocaine-, cue- and stress-induced reinstatement.<sup>8,47</sup> In addition, inactivation of PFC and basolateral amygdala (BLA)

abolishes context-induced reinstatement<sup>48</sup> and inactivation of the dopaminergic neurons in the VTA inhibits all modalities of reinstated drug-seeking so far tested.<sup>49</sup> Along these lines, while dopamine afferents to the PFC are critical for both cocaine- and cue-induced reinstatement, only DA input to the shell subcompartment of the NA (NAshell) is necessary for reinstated behaviour, while DA receptor blockade in the core subcompartment (NAcore) does not alter reinstated cocaine-seeking.<sup>50</sup> In addition to the series circuit termed the final common pathway, including PFC, VTA, NAcore and VP<sup>6</sup>, there are other key cortical regions found to have involvement in potentiating or inhibiting relapse. The orbitofrontal cortex (OFC) has glutamatergic projections to NAcore that are key for inhibiting various types of impulsive responses that may lead to addiction,<sup>51</sup> including response inhibition,<sup>52</sup> reward and punishment sensitivity, emotional decision-making and reversal learning.<sup>53–55</sup> In addition to cortical areas, the orexin neurons in the lateral hypothalamus (LH) project to the VTA,

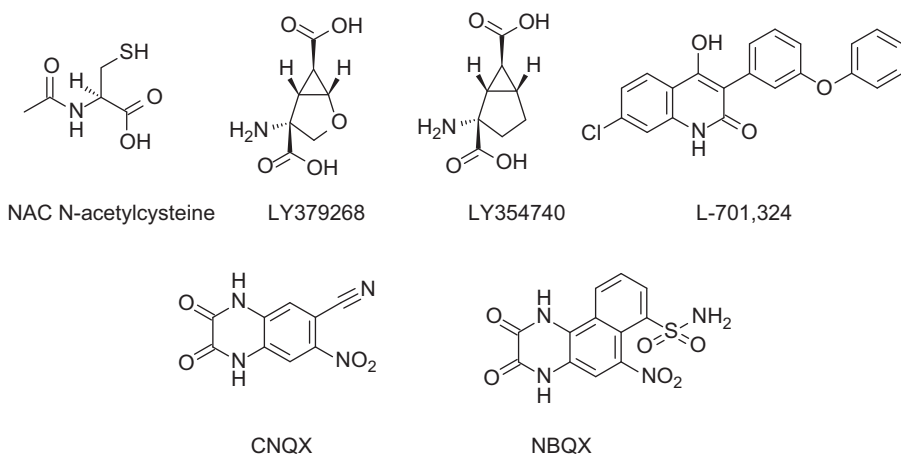


**Figure 14.4** A schematic of the neurocircuitry involved in drug abuse and relapse. [PFC=prefrontal cortex; OFC=orbitofrontal cortex; Ext AMG=extended amygdala; BLA=basolateral amygdala; LH=lateral hypothalamus; NAcore=nucleus accumbens core; Medial Dorsal Thal=medial dorsal thalamus; VTA=ventral tegmental area; VP=ventral pallidum].

and modulate glutamatergic transmission in VTA DA neurons.<sup>56</sup> See Figure 14.4 for a schematic of the reward circuitry involved in addiction.

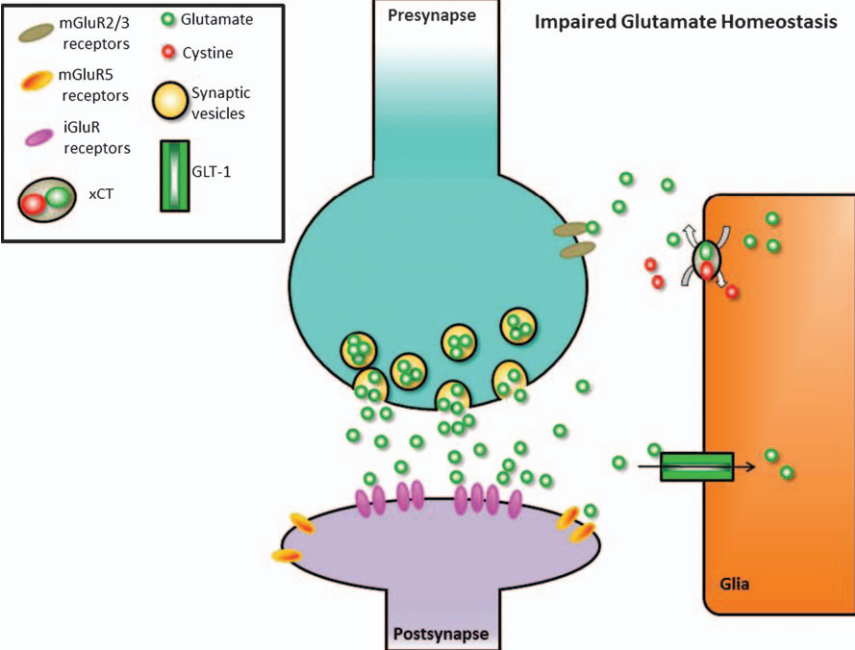
Within this reward circuitry, a dysregulation of glutamatergic mechanisms has been hypothesized such that alterations in glutamate homeostasis contribute to relapse of drug-seeking behaviour. Extensive evidence indicates that chronic exposure to cocaine or nicotine leads to an enduring decrease in the expression of the system xc- (cystine-glutamate exchanger) catalytic subunit xCT, and glutamate transporters GLT-1 and GLAST in the NA, and consequently a decrease in extra-cellular glutamate levels (see Figure 14.6a for a schematic of impaired glutamate homeostasis).<sup>57–63</sup> The decreased basal extra-cellular glutamate levels in the NA reduces tone on pre-synaptic inhibitory mGluR2/3 receptors, and this basal tone provides negative feedback and control of synaptic glutamate release probability. However, in the absence of glutamatergic tone onto mGluR2/3 associated with reduced cystine-glutamate exchange, excessive glutamate transmission can ensue.<sup>64</sup> Thus, presentation of drug or a drug-associated cue leads to a potentiated response in a preclinical model of relapse, and this drug seeking is associated with elevated extra-cellular glutamate derived from synaptic activity in the prefrontal-NA pathway.<sup>65</sup>

Because impaired glutamate homeostasis has been linked to the reinstatement of drug-seeking behaviour, attempts have been made to repair the imbalance between glial and synaptic glutamate via various drugs that target dysregulated proteins of the tripartite synapse (pre-synapse, post-synapse and astroglia). Of these pharmacotherapies, one is the commonly prescribed antibiotic ceftriaxone, and another is the nutritional supplement *N*-acetylcysteine (NAC). After chronic cocaine or nicotine use, both xCT and the glial glutamate transporter GLT-1 are down-regulated. Ceftriaxone and NAC (Figure 14.5) have been found to restore both xCT and GLT-1 levels, and thus inhibit reinstatement of cocaine-seeking behaviour in rats.<sup>62</sup> As well, NAC has been

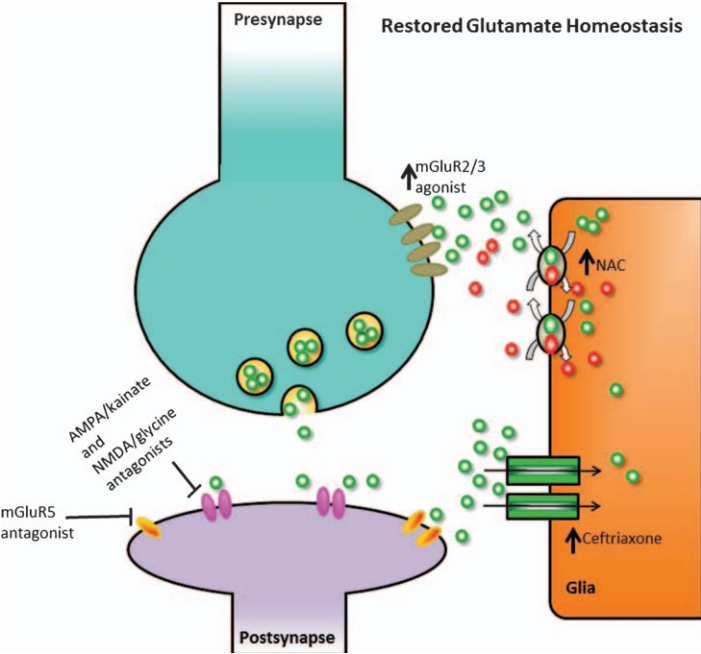


**Figure 14.5** Drugs targeting glutamergic signalling.

(a)



(b)



shown to restore synaptic plasticity (*e.g.* long-term potentiation and depression) in NA that is impaired following chronic cocaine self-administration.<sup>66</sup> In humans, NAC decreases cigarette use<sup>67</sup> and cocaine craving and symptoms of withdrawal in cocaine-dependent individuals.<sup>68</sup> In addition to targeting glial proteins involved in glutamate homeostasis, both pre-synaptic and post-synaptic metabotropic glutamate receptors have been targeted preclinically to inhibit relapse. As described above, reduced glial release of glutamate onto pre-synaptic mGluR2/3 is decreased following chronic cocaine use, thereby decreasing inhibitory tone on pre-synaptic glutamate release.<sup>69</sup> As well, systemic and intra-accumbens application of an mGluR2/3 agonist inhibits the reinstatement of cocaine and food seeking by reducing pre-synaptic glutamate release.<sup>70,71</sup> Post-synaptically, inhibition of mGluR5 receptors inhibits cue- and cocaine-induced reinstatement of cocaine-seeking behaviour, as well as nicotine self-administration.<sup>72,73</sup> In addition to metabotropic glutamate receptors, antagonism of post-synaptic ionotropic glutamate receptors (AMPA, NMDA and kainate receptors) has been found to inhibit reinstated drug-seeking. Specifically, the AMPA/kainate receptor antagonists CNQX and NBQX, and the NMDA/glycine site antagonist L-701,324 (Figure 14.5), significantly inhibited cue-induced reinstatement of cocaine-seeking behaviour.<sup>74</sup> See Figure 14.6b for a schematic of restored glutamate homeostasis via the pharmacological targets described above.

### 14.4.1 Alcohol

Historically, ethanol was thought to exert its effects on the brain via GABAergic mechanisms. More recent work has revealed that adaptations induced by alcohol on glutamatergic signalling may be targeted to potentially ameliorate alcohol addiction. For example, repeated ethanol up-regulates the NR1, NR2A and NR2B subunits of NMDA receptors in cerebral cortex and hippocampus.<sup>75–77</sup> Additionally, activation of NA mGluR5 receptors is necessary for ethanol self-administration in animals bred for high ethanol consumption.<sup>78</sup> mGluR2/3 receptors have also been implicated in ethanol self-administration and cue-induced reinstatement of ethanol seeking, as both behaviours were suppressed following systemic mGluR2/3 agonist (LY379268,

---

**Figure 14.6** A schematic of the pharmacological targeting of glutamate homeostasis in the treatment of addiction. (A) Impaired glutamate homeostasis after chronic use of an addictive drug. Expression of both xCT and GLT-1 is decreased, which results in decreased basal levels of extra-synaptic glutamate. As well, decreased xCT results in decreased glutamatergic tone on inhibitory mGluR2/3 receptors, which in turn increases glutamatergic transmission and activation of post-synaptic ionotropic glutamate (*i.e.* AMPA, NMDA and kainite receptors) and mGluR5 receptors. (B) Restoration of glutamate homeostasis following administration of ceftriaxone and/or NAC, which normalize the balance between synaptic and extra-synaptic glutamate levels. As well, administration of these agents inhibits drug seeking.

Figure 14.5) administration.<sup>79</sup> Although chronic ethanol affects glutamatergic mechanisms, other research has found that mGluR2/3 agonists and mGluR5 antagonists do not inhibit convulsive activity during withdrawal from chronic ethanol exposure, and thus these compounds may not be useful pharmacotherapies to reduce the central nervous system hyperexcitability experienced during alcohol withdrawal.<sup>80</sup> The mGluR5 antagonist MPEP, however, decreases ethanol consumption in animal models of binge drinking.<sup>81</sup>

Acamprosate is a drug used to treat alcoholism that was originally developed in Europe in the 1980s for this purpose. Although originally thought to act via GABAergic transmission, other work has elucidated glutamatergic mechanisms through which acamprosate exerts its effects. Specifically, research has found that locally applied acamprosate decreases the excitation of cortical neuronal firing evoked by application of L-glutamate *in vivo*, and inhibits excitatory postsynaptic potentials (EPSPs) evoked by glutamate in hippocampal tissue slices. Taken together, it appears that acamprosate may act as an antagonist of excitatory neurotransmission, thus decreasing neuronal excitability,<sup>82</sup> although other electrophysiological work is inconsistent with this conclusion.<sup>83</sup> Despite these inconsistencies, there is an overall consensus that acamprosate acts as an NMDA receptor modulator.<sup>84</sup>

## 14.4.2 Nicotine

Tobacco smoking is one of the leading preventable causes of premature death,<sup>85</sup> and alcohol use is linked to various health problems. Nicotine (Figure 14.1), the primary active alkaloid in tobacco, is generally accepted as being responsible for maintaining smoking behaviour.<sup>86,87</sup> The reinforcing property of nicotine has been demonstrated in laboratory studies with rodents,<sup>88–91</sup> non-human primates<sup>92</sup> and humans<sup>93</sup> using the intravenous self-administration paradigm. Nicotine engenders self-administration behaviour through activation of high-affinity  $\beta 2$  subunit-containing nicotinic cholinergic receptors localized on dopamine cell bodies in the ventral tegmental area (VTA), and by altering glutamatergic and GABAergic tone in the VTA;<sup>94</sup> the net result is increased levels of extra-cellular dopamine in the nucleus accumbens.<sup>95–98</sup> Nicotine also exerts its actions on a number of neurochemical systems that may contribute to its addictive properties, including brainstem cholinergic, noradrenergic and serotonergic, and GABAergic nuclei, in addition to its actions on dopaminergic neurotransmission.<sup>99</sup> A variety of pharmacotherapies have been employed in order to enhance efforts to discontinue tobacco use, including the use of nicotine-replacement therapy,<sup>100,101</sup> and the nicotinic partial agonist varenicline.<sup>102,103</sup> However, relapse rates continue to be high, indicating that alternatives are needed to treat tobacco dependence.

Although the majority of work on glutamatergic mechanisms in addiction has been with cocaine (described previously and in Section 14.5.3), research on the effects of nicotine on glutamate homeostasis is currently underway. Although less is known about the effects of chronic nicotine use on glutamate homeostasis, similar changes in key proteins associated with glutamate



homeostasis have been found following chronic nicotine self-administration. Importantly, 24 hours after nicotine self-administration there are changes in xCT, GLT1 and mGluR2/3 content and/or function.<sup>67,104</sup> Similar to the effects of chronic cocaine self-administration, levels of xCT and GLT1 were decreased in NA. As well, NAC was effective at decreasing nicotine use in cigarette smokers when taken chronically.<sup>67</sup> As a possible pharmacotherapeutic agent in nicotine withdrawal, the mGluR2/3 agonist LY354740 (Figure 14.5) decreases the sensorimotor reactivity associated with nicotine withdrawal in rats.<sup>105</sup>

### 14.4.3 Cocaine and Heroin

Chronic use of cocaine has been found to produce enduring neuroadaptations in the corticostriatal brain circuitry involved in the plasticity of learning and behaviour,<sup>3</sup> including: (1) increases in post-synaptic density 95 protein, filamentous (F)-actin and AMPA ionotropic glutamate receptors (iGluRs), and increased or decreased Homer levels; (2) decreases in glial GLT1<sup>62</sup> and xCT,<sup>106</sup> as well as pre-synaptic mGluR2/3s,<sup>107</sup> due to decreased xCT and thus decreased tone on these receptors; (3) marked deficits in long-term potentiation and long-term depression in NA following withdrawal from chronic cocaine self-administration;<sup>66</sup> and (4) morphological changes in dendritic spines of medium spiny neurons (MSNs) of the NA<sup>108</sup> (for a review, see ref. 3; see Figure 14.6a). These morphological adaptations are thought to impair the ability of the NA to process information from cortical afferents that adaptively regulate reward-seeking behaviours, and thus contribute to addiction-related behaviours such as relapse. MSNs in NA receive glutamatergic input from limbic and cortical regions, including basolateral amygdala (BLA; involved in the processing of emotionally salient stimuli) and PFC (involved in the processing of executive and motor planning). The MSNs integrate information from these regions, as well as from dopamine cells in the VTA, and thereby contribute to the execution of goal-directed behaviour.<sup>109,110</sup> Pharmacotherapeutic agents found to inhibit relapse of cocaine-seeking behaviour include activating group II mGluRs, inhibiting group I mGluRs, inhibiting iGluRs, and increasing the function of xCT and GLT1 via NAC and ceftriaxone (described previously).

Although less is known, research has been conducted to examine the effects of chronic heroin (Figure 14.1) on glutamate homeostasis. Previous preclinical research has found that heroin- or cue-induced heroin reinstatement increased NAc core extra-cellular glutamate. As well, this increase is abolished by inhibiting NAc core synaptic transmission with tetrodotoxin, and the reinstatement of heroin-seeking behaviour is inhibited by AMPA/kainate iGluR inhibition via CNQX micro-infusion into NAc core or the systemic administration of NAC.<sup>111</sup> These results suggest that pharmacotherapies found to target the glutamatergic system and inhibit cocaine relapse may also be useful as pharmacotherapies to inhibit heroin relapse.

## 14.5 Opioid System as a Potential Pharmacotherapeutic Target

The opioid system is comprised of three receptors, including mu ( $\mu$ ), kappa ( $\kappa$ ) and delta ( $\delta$ ). These receptors are activated by endogenous peptides, and are recruited to the cell surface in response to natural rewarding stimuli and drugs of abuse. Mu opioid receptors have been mainly implicated in drug reinforcement and dependence, as  $\mu$  receptor knockout mice are insensitive to morphine suggesting that these receptors are the primary target for opiate drugs *in vivo*.<sup>112</sup> Additionally, more recent research suggests that  $\kappa$  opioid receptors may be a useful therapeutic target.

When  $\kappa$  opioid receptors were first identified, there was interest in developing non-addictive pain medications, since the activation of  $\mu$  opioid receptors stimulates reward pathways. Early attempts to develop antagonists of  $\kappa$  opioid receptors, however, led to compounds with side-effects such as dysphoria, diuresis and constipation. Since that time,  $\kappa$ -opioid antagonists have shown promise as antidepressants,<sup>113</sup> and may be useful therapeutic tools in increasing stress resilience; thus they have been proposed in treating certain forms of anxiety, depression and substance abuse disorders, as hypersensitivity to stress intensifies each of these conditions. One caveat, however, is that  $\kappa$ -opioid antagonists have very long durations of action, and it is unclear what mechanisms underlie this characteristic.<sup>114</sup>

### 14.5.1 Alcohol

The opioid system has been implicated in the treatment of alcohol addiction. At a preclinical level of analysis,  $\mu$  opioid receptors in the VP have been implicated in the regulation of voluntary ethanol consumption.<sup>115</sup> As well, the  $\mu$  opioid receptor antagonist naltrexone reduces ethanol intake.<sup>81</sup> Interestingly,  $\delta$  opioid receptor knockout mice show increased ethanol self-administration, and ethanol intake reduces characteristic high anxiety levels in these animals.<sup>116</sup> Also,  $\delta$  receptor antagonists decrease cue- and stress-induced reinstatement of ethanol-seeking behaviour in rats, suggesting a role of these receptors in relapse.<sup>117</sup> Taken together, these results suggest an interesting role of the  $\delta$  opioid receptor in alcohol addiction, and targeting of this receptor may yield important treatment options in alcohol addiction.

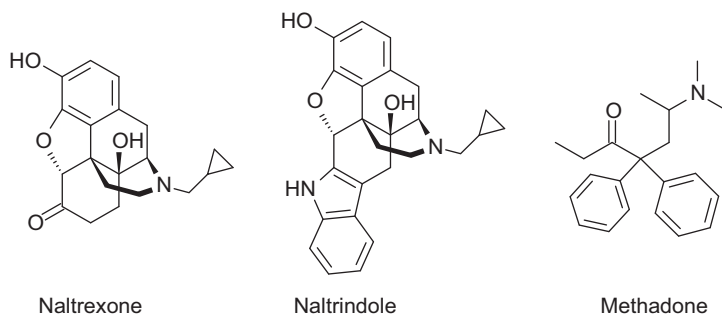
Clinical utility of opioid antagonists has been shown in alcohol-dependent subjects. In humans, extended-release naltrexone (XR-NTX, Figure 14.6) has been shown to prolong abstinence from alcohol and reduce the number of drinking days in patients.<sup>118</sup> As well, patients taking acamprosate or disulfiram were more likely to discontinue treatment than patients taking XR-NTX,<sup>119</sup> indicating that this may be a viable treatment strategy with improved compliance. Although efficacy of short-acting, oral naltrexone is somewhat controversial, it appears that long-acting, injectable naltrexone is both efficacious and well tolerated.<sup>120</sup>

### 14.5.2 Nicotine

In addition to insensitivity to morphine,  $\mu$  receptor knockout mice also do not develop nicotine place preference, thus indicating  $\mu$  receptors in the rewarding effects of nicotine. These mice also did not develop typical withdrawal syndrome when withdrawal was precipitated with the nicotinic antagonist mecamylamine.<sup>121</sup> As well,  $\mu$  opioid receptors and CREB activation are required for nicotine reward, as an injection of naloxone (an opioid receptor antagonist) blocks both CREB phosphorylation and nicotine reward in a conditioned place preference task in mice.<sup>122</sup> Furthermore, both naloxone and the  $\kappa$  opioid receptor agonist (U50, 488) dose-dependently attenuated nicotine self-administration in rats, and the specific  $\delta$  opioid antagonist (naltrindole, Figure 14.7) did not alter nicotine self-administration.<sup>123</sup> Taken together, these results suggest a role of the  $\kappa$  and  $\mu$  opioid receptors in nicotine reward and withdrawal. In clinical trials, the administration of naltrexone (a single oral dose) attenuates the post-cigarette craving rating, as well as desire to smoke and the total number of cigarettes smoked in nicotine-dependent human subjects.<sup>124</sup>

### 14.5.3 Cocaine, Heroin and Methamphetamine

As mentioned previously, naltrexone (Figure 14.7) is a non-addictive opioid antagonist that inhibits relapse to use of opioids such as heroin by blocking opioid receptors. Although this drug makes it impossible to return to use of opioids, it has poor reception among individuals with a heroin addiction due to compliance issues.<sup>125</sup> Thus, other pharmacotherapies are needed to improve compliance and reduce relapse rates among opioid addicts. In the treatment of cocaine, use of naltrexone has been met with mixed results. Preclinically, naltrexone was found to decrease cocaine self-administration in monkeys,<sup>126</sup> but increase cocaine self-administration in rats.<sup>127</sup> In humans, naltrexone attenuated cocaine-induced increases in the subjective rating of cocaine value and unpleasant feelings following an intravenous cocaine crash,<sup>128</sup> while another study reported that naltrexone did not affect the subjective or physiological effects of intravenous cocaine.<sup>129</sup> Clinically, naltrexone was found to be more



**Figure 14.7** Drugs that target the opioid system.

effective than control treatments in reducing cocaine use when paired with relapse prevention treatment.<sup>130</sup> Despite conflicting results at both the pre-clinical and clinical levels of analysis, it appears that naltrexone might have value as an effective pharmacotherapy in the treatment of cocaine addiction.

For opioid dependence, methadone maintenance therapy (MMT) has been an effective treatment for individuals with a heroin addiction, and has been shown to be a safe and effective treatment for over 40 years.<sup>131</sup> Methadone (Figure 14.7), a slow-acting opioid agonist with a long half-life, is administered orally. Thus, methadone does not produce sharp increases in plasma levels found with an injection of heroin, and once-daily administered methadone produces relatively constant plasma opioid levels, thereby attenuating cravings as well as withdrawal symptoms.

Preclinically, evidence suggests a role for naltrexone in attenuating methamphetamine-induced behavioural sensitization<sup>132</sup> and cue-induced reinstatement of methamphetamine-seeking in a model of relapse.<sup>133</sup> Although initial studies in humans have suggested that a NAC plus naltrexone combination therapy failed to treat methamphetamine dependence,<sup>134</sup> it was found that naltrexone reduces the reinforcing effects of amphetamine in humans,<sup>135,136</sup> suggesting that this may be a potential pharmacotherapy in the treatment of amphetamine dependence.

## 14.6 Other Pharmacotherapies for Drug Dependence

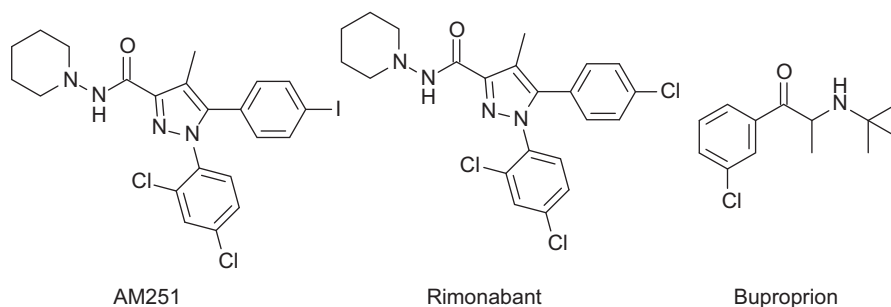
Thus far, this chapter has covered some of the key neurobiological systems implicated in addiction that have been targeted for the development of pharmacotherapies, but other drug targets are briefly described below that also show promise as pharmacotherapies for drug addiction.

### 14.6.1 Orexin

Orexin, a neurotransmitter involved in arousal and feeding behaviour, has been implicated in addiction. Evidence suggests that DA neurons in the VTA are influenced by orexin/hypocretin-containing neurons projecting from the lateral hypothalamus (LH) to the VTA (see Figure 14.4). Specifically, application of orexin A *in vitro* was found to potentiate NMDA-mediated neurotransmission in VTA DA neuron synapses. As well, Orexin1 (Ox1) receptor antagonists were found to block cocaine behavioural sensitization.<sup>137</sup> Antagonizing the Orexin1 (Ox1) receptor was also found to inhibit cue- and context- but not cocaine-induced cocaine seeking.<sup>138</sup> Thus, the Ox1 receptor may be an important therapeutic target in treating addiction.

### 14.6.2 Cannabinoids

There is compelling evidence that the endocannabinoid system may be a fruitful avenue for pharmacotherapeutic intervention of drug dependence. Specifically,



**Figure 14.8** Other pharmacotherapies for drug dependence.

cannabinoid-1 (CB-1) receptor antagonists, such as AM251 or Rimonabant (Figure 14.8), may be useful pharmacotherapies for various drugs of abuse. CB-1 receptor antagonists act either to block the rewarding effects of drugs such as THC, or to enhance extinction learning and block the ability of conditioned cues to reinstate drug-seeking behaviour.<sup>139</sup> AM251, when administered into NA, inhibits methamphetamine intra-accumbal self-administration.<sup>140</sup>

### 14.6.3 Replacement Therapies

In addition to potentially non-addictive pharmacotherapies developed to treat addiction, another important avenue of treatment is agonist replacement therapy (mentioned previously). The agonist-like model for drug dependence hypothesizes that medication with properties similar to that of the abused drug, but with less abuse potential, will stabilize neurochemistry and behaviour and will in turn reduce drug use. For example, the nicotine patch and the  $\mu$  agonist methadone are classic replacement therapies for nicotine and heroin addiction. An agonist replacement therapy for stimulants is not FDA approved; however, clinical trials have been conducted to examine the ability of two drugs prescribed in the treatment of attention deficit hyperactivity disorder (ADHD), d-amphetamine and methylphenidate, to decrease methamphetamine use.<sup>141</sup> Indeed, d-amphetamine substitutes for cocaine<sup>142</sup> and methamphetamine<sup>143</sup> in a drug discrimination paradigm.

### 14.6.4 Antidepressants

One treatment of interest in nicotine addiction is the monoamine uptake blocker bupropion.<sup>144</sup> Bupropion (Figure 14.8) is an antidepressant medication that has been found to increase success rates in smoking cessation. Recent evidence suggests that genetic variants in the serotonin transporter influence the efficacy of bupropion in smoking cessation,<sup>145</sup> thus pharmacogenetics may be useful in determining individual differences in response to this medication as an efficacious pharmacotherapy. Bupropion has also been indicated in the

treatment of methamphetamine dependence.<sup>146</sup> Preclinically, bupropion has been shown to decrease methamphetamine self-administration in rats.<sup>147</sup>

### 14.6.5 Vaccines

Vaccinations for addiction have been developed. Drugs of abuse are small molecules incapable of eliciting an antibody response on their own. Thus, these molecules need to be conjugated to an immunogenic protein or some carrier to elicit an antibody response, and a linker that allows conjugation to the carrier is attached covalently to the drug molecule.<sup>148</sup> Specifically, anti-nicotine vaccines (*e.g.* NicVax, NicQb and TA-Nic) are in advanced clinical trials. These vaccines are irreversible and need booster injections, and they interact with the drug in the blood rather than with the receptor in the brain by binding to the drug.<sup>149</sup> For cocaine, the only vaccine that has entered human clinical trials employs succinyl norcocaine attached to recombinant cholera toxin B. In both anti-nicotine and anti-cocaine vaccines, there are individual discrepancies in the amount of antibody concentrations achieved, and high responder subjects are better able to inhibit drug use.<sup>150</sup>

### 14.6.6 Pharmacogenetics

An attractive new avenue of research is to incorporate individual genetic differences that are predictive of both the therapeutic effects and side-effects of a drug. Pharmacogenetics may increase the sensitivity and predictive validity of clinical trials by reducing variability in drug responses. By using this technique, it is suggested that a more homogeneous sample can be recruited, which can in turn decrease variance and increase medication response. As well, identification of subjects with an increased likelihood of adverse side-effects is an important application of pharmacogenetics. Because pharmacogenetics is relatively new, its clinical utility has yet to be fully shown and false positives are of concern with this approach.

Diverse targets for treatment of drug dependence have been identified, and individual differences are likely to exist in how efficacious these various treatments that have unique targets will be. Thus, personalized pharmacotherapies are of great potential importance, as not only do genetic factors influence the onset and severity of addiction, but they are also likely to influence the efficacy of various treatment strategies.<sup>151,152</sup> Although a relatively new field of research, pharmacogenetics is a tool to examine individual differences in drug metabolism (pharmacokinetics and pharmacodynamics), and thus may help predict responses to medication, side-effects and appropriate dosing strategies.<sup>153</sup> As well, there is a constellation of underlying neural pathways and processes involved in the rewarding effects of drugs of abuse, and identifying one pharmacotherapeutic target that will be effective for every individual with a substance-use disorder is not likely. As a new and promising field,

pharmacogenetics thus far has provided some evidence for genetic differences in response to pharmacotherapies for drugs of abuse.

Genetic predispositions have been identified for alcoholism. Specifically, genes for the enzymes of alcohol metabolism influence alcohol drinking behaviour and risk for developing alcohol addiction. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are liver enzymes responsible for the oxidative metabolism of alcohol,<sup>154</sup> and are polymorphic in humans. Alcoholics show lower frequencies of the ADH2\*2, ADH3\*1 and ALDH2\*2 allele than non-alcoholics, thus indicating that genetic variation in both ADH and ALDH influences drinking behaviour and risk for developing an addiction to alcohol.<sup>155</sup> In addition to work on genetics of the enzymes responsible for metabolism of alcohol, clinical trials suggest that a family history of alcoholism predicts response to naltrexone for treatment of alcohol dependence.<sup>156</sup> As well, results from laboratory studies support this possibility, with both subjective effects of alcohol<sup>157</sup> and alcohol self-administration.<sup>158</sup>

Pharmacogenetic modulators of opioid therapy have been identified.<sup>159</sup> Specifically, codeine is ineffective in those with genetic inactivity of cytochrome P450 (CYP) 2D6,<sup>160</sup> and is toxic in patients with ultrarapid CYP2D6 metabolism.<sup>161</sup> As well, a single nucleotide polymorphism A118G of the  $\mu$  opioid receptor gene has been linked to decreased potency of morphine.<sup>162</sup> Indeed, genetic influence can modify drug interactions and thus modify efficacy of opioid therapy. Clinically, however, pharmacogenetics of opioids is limited to codeine, as poor metabolizers of debrisoquine/sparteine should not be administered codeine due to possible drug interactions and toxicity.<sup>163</sup> In addition, other research has examined alcohol-dependent individuals with two specific single nucleotide polymorphisms (SNP) of the gene encoding the  $\mu$  opioid receptor, including the Asn40Asp and Ala6Val polymorphisms.<sup>164</sup> In this study, naltrexone-treated individuals with either one or two copies of the Asp40 allele had significantly lower relapse rates than those who were homozygous for the Asn40 allele when measured over a 12-week treatment period. As well, no differences in relapse rates were found between the two genotype groups when given placebo, indicating that this polymorphism may be useful in identifying alcohol-dependent individuals that may be more likely to respond to naltrexone treatment.

## 14.7 Conclusions

Addiction is a chronic, relapsing disorder that warrants treatment. As such, addictive disorders should be included along with other disorders that need long-term or life-long treatment. Compliance with treatment remains a primary issue in the treatment of addictive disorders. As well, both behavioural and pharmacotherapeutic treatment in combination is likely to be a more effective treatment strategy than either one alone. Although quite a few targets have been identified in the treatment of various drug dependencies, it is important to assess treatment for the individual patient to maximize efficacy and compliance.



Along these lines, pharmacogenetics may be a promising advance in the future of drug development, in both decreasing cost and increasing successful treatment of addiction. This may be an especially profitable approach given the diverse number of potential targets for treating addiction that have emerged from studies in animal models of the neurological adaptations associated with chronic drug use.

## References

1. D. A. Kessler, S. L. Natanblut, J. P. Wilkenfeld, C. C. Lorraine, S. L. Mayl, I. B. Bernstein and L. Thompson, *J. Pediatr.*, 1997, **130**, 518–524.
2. P. W. Kalivas and C. O'Brien, *Neuropsychopharmacology*, 2008, **33**, 166–180.
3. P. W. Kalivas, *Nat. Rev. Neurosci.*, 2009, **10**, 561–572.
4. E. Carboni, A. Imperato, L. Perezzi and G. Di Chiara, *Neuroscience*, 1989, **28**, 653–661.
5. E. Carboni, A. Silvagni, M. T. Rolando and G. Di Chiara, *J. Neurosci.*, 2000, **20**, RC102.
6. P. W. Kalivas and N. D. Volkow, *Am. J. Psychiatry*, 2005, **162**, 1403–1413.
7. N. D. Volkow, J. S. Fowler, G. J. Wang, R. Baler and F. Telang, *Neuropharmacology*, 2009, **56**(1), 3–8.
8. K. McFarland and P. W. Kalivas, *J. Neurosci.*, 2001, **21**, 8655–8663.
9. A. Bowirrat and M. Oscar-Berman, *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 2005, **132B**, 29–37.
10. T. Hillemeier, H. Frieeling, T. Hartl, J. Wilhelm, J. Kornhuber and S. Bleich, *J. Psychiatr. Res.*, 2009, **43**, 388–392.
11. L. L. Howell and K. M. Wilcox, *J. Pharmacol. Exp. Ther.*, 2001, **298**, 1–6.
12. J. Bergman, J. B. Kamien and R. D. Spealman, *Behav. Pharmacol.*, 1990, **1**, 355–363.
13. S. B. Caine, G. F. Koob, L. H. Parsons, B. J. Everitt, J. C. Schwartz and P. Sokoloff, *NeuroReport*, 1997, **8**, 2373–2377.
14. P. Sokoloff, B. Giros, M. P. Martres, M. L. Bouthenet and J. C. Schwartz, *Nature*, 1990, **347**, 146–151.
15. P. Sokoloff, B. Giros, M. P. Martres, M. Andrieux, R. Besancon, C. Pilon, M. L. Bouthenet, E. Souil and J. C. Schwartz, *Arzneimittelforschung*, 1992, **42**, 224–230.
16. S. B. Caine and G. F. Koob, *Science*, 1993, **260**, 1814–1816.
17. C. A. Heidbreder, E. L. Gardner, Z. X. Xi, P. K. Thanos, M. Mugnaini, J. J. Hagan and C. R. Ashby, Jr, *Brain Res. Brain Res. Rev.*, 2005, **49**, 77–105.
18. R. Spanagel, *Physiol. Rev.*, 2009, **89**, 649–705.
19. M. Heilig, A. Thorsell, W. H. Sommer, A. C. Hansson, V. A. Ramchandani, D. T. George, D. Hommer and C. S. Barr, *Neurosci. Biobehav. Rev.*, 2010, **35**, 334–344.

20. F. Weiss, M. T. Lorang, F. E. Bloom and G. F. Koob, *J. Pharmacol. Exp. Ther.*, 1993, **267**, 250–258.
21. N. K. Kamdar, S. A. Miller, Y. M. Syed, R. Bhayana, T. Gupta and J. S. Rhodes, *Psychopharmacology (Berl.)*, 2007, **192**, 207–217.
22. T. P. Laine, A. Ahonen, P. Torniainen, J. Heikkilä, J. Pyhtinen, P. Rasanen, O. Niemela and M. Hillbom, *Mol. Psychiatry*, 1999, **4**, 189–191, 104–185.
23. V. Vengeliene, F. Leonardi-Essmann, S. Perreau-Lenz, P. Gebicke-Haerter, K. Drescher, G. Gross and R. Spanagel, *Faseb J*, 2006, **20**, 2223–2233.
24. P. K. Thanos, J. M. Katana, C. R. Ashby, Jr, M. Michaelides, E. L. Gardner, C. A. Heidbreder and N. D. Volkow, *Pharmacol. Biochem. Behav.*, 2005, **81**, 190–197.
25. J. Grabowski, J. D. Roache, J. M. Schmitz, H. Rhoades, D. Creson and A. Korszun, *J. Clin. Psychopharmacol.*, 1997, **17**, 485–488.
26. N. D. Volkow, Y. S. Ding, J. S. Fowler, G. J. Wang, J. Logan, J. S. Gatley, S. Dewey, C. Ashby, J. Liebermann, R. Hitzemann and A. P. Wolf, *Arch. Gen. Psychiatry*, 1995, **52**, 456–463.
27. N. D. Volkow, G. J. Wang, J. S. Fowler, S. J. Gatley, J. Logan, Y. S. Ding, R. Hitzemann and N. Pappas, *Am. J. Psychiatry*, 1998, **155**, 1325–1331.
28. K. D. Burris, T. F. Molski, C. Xu, E. Ryan, K. Tottori, T. Kikuchi, F. D. Yocca and P. B. Molinoff, *J. Pharmacol. Exp. Ther.*, 2002, **302**, 381–389.
29. C. Eroglu, N. J. Allen, M. W. Susman, N. A. O'Rourke, C. Y. Park, E. Ozkan, C. Chakraborty, S. B. Mulinyawe, D. S. Annis, A. D. Huberman, E. M. Green, J. Lawler, R. Dolmetsch, K. C. Garcia, S. J. Smith, Z. D. Luo, A. Rosenthal, D. F. Mosher and B. A. Barres, *Cell*, 2009, **139**, 380–392.
30. L. Karila, A. Weinstein, H. J. Aubin, A. Benyamina, M. Reynaud and S. L. Batki, *Br. J. Clin. Pharmacol.*, 2010, **69**, 578–592.
31. L. P. Dwoskin and P. A. Crooks, *Biochem. Pharmacol.*, 2002, **63**, 89–98.
32. N. M. Neugebauer, S. B. Harrod, D. J. Stairs, P. A. Crooks, L. P. Dwoskin and M. T. Bardo, *Eur. J. Pharmacol.*, 2007, **571**, 33–38.
33. J. S. Beckmann, K. B. Siripurapu, J. R. Nickell, D. B. Horton, E. D. Denehy, A. Vartak, P. A. Crooks, L. P. Dwoskin and M. T. Bardo, *J. Pharmacol. Exp. Ther.*, 2010, **335**, 841–851.
34. A. C. Meyer, D. B. Horton, N. M. Neugebauer, T. E. Wooters, J. R. Nickell, L. P. Dwoskin and M. T. Bardo, *Neuropharmacology*, 2011, **61**, 849–856.
35. F. J. Vocci and N. M. Appel, *Addiction*, 2007, **102**(1), 96–106.
36. K. G. Commons, *Brain Res.*, 2010, **1314**, 175–182.
37. T. Hokfelt, B. Pernow and J. Wahren, *J. Intern. Med.*, 2001, **249**, 27–40.
38. Y. Nakaya, T. Kaneko, R. Shigemoto, S. Nakanishi and N. Mizuno, *J. Comp. Neurol.*, 1994, **347**, 249–274.
39. T. M. Ballard, S. Sanger and G. A. Higgins, *Eur. J. Pharmacol.*, 2001, **412**, 255–264.

40. N. M. Rupniak and M. S. Kramer, *Trends Pharmacol. Sci.*, 1999, **20**, 485–490.
41. M. Munoz and R. Covenas, *Curr. Med. Chem.*, 2011, **18**, 1820–1831.
42. L. Leggio, S. Cardone, A. Ferrulli, G. A. Kenna, M. Diana, R. M. Swift and G. Addolorato, *Curr. Pharm. Des.*, 2010, **16**, 2159–2118.
43. J. R. Schank, C. L. Pickens, K. E. Rowe, K. Cheng, A. Thorsell, K. C. Rice, Y. Shaham and M. Heilig, *Psychopharmacology (Berl.)*, 2011, **218**(1), 111.
44. P. Steensland, J. A. Simms, C. K. Nielsen, J. Holgate, J. J. Bito-Onon and S. E. Bartlett, *PLoS One*, 2010, **5**(9), p. ii, e12527.
45. T. L. Ripley, C. A. Gadd, C. De Felipe, S. P. Hunt and D. N. Stephens, *Neuropharmacology*, 2002, **43**, 1258–1268.
46. R. Maldonado, D. Girdlestone and B. P. Roques, *Neurosci. Lett.*, 1993, **156**, 135–140.
47. K. McFarland, C. C. Lapish and P. W. Kalivas, *J. Neurosci.*, 2003, **23**, 3531–3537.
48. T. Hillemacher, H. Frieling, K. Bayerlein, J. Wilhelm, J. Kornhuber and S. Bleich, *J. Neural. Transm.*, 2007, **114**, 151–154.
49. P. Di Ciano and B. J. Everitt, *Eur. J. Neurosci.*, 2004, **19**, 1661–1667.
50. N. Schintu, L. Frau, M. Ibba, P. Caboni, A. Garau, E. Carboni and A. R. Carta, *Eur. J. Neurosci.*, 2009, **29**, 954–963.
51. H. de Wit, *Addict Biol.*, 2009, **14**, 22–31.
52. C. A. Winstanley, *Ann. NY Acad. Sci.*, 2007, **1121**, 639–655.
53. M. L. Kringelbach and E. T. Rolls, *Prog. Neurobiol.*, 2004, **72**, 341–372.
54. R. Cools, L. Clark, A. M. Owen and T. W. Robbins, *J. Neurosci.*, 2002, **22**, 4563–4567.
55. X. Liu, D. K. Powell, H. Wang, B. T. Gold, C. R. Corbly and J. E. Joseph, *J. Neurosci.*, 2007, **27**, 4587–4597.
56. G. Aston-Jones, R. J. Smith, D. E. Moorman and K. A. Richardson, *Neuropharmacology*, 2009, **56**(1), 112–121.
57. D. A. Baker, K. McFarland, R. W. Lake, H. Shen, S. Toda and P. W. Kalivas, *Ann. NY Acad. Sci.*, 2003, **1003**, 349–351.
58. R. C. Pierce, K. Bell, P. Duffy and P. W. Kalivas, *J. Neurosci.*, 1996, **16**, 1550–1560.
59. W. J. Berglind, T. W. Whitfield, Jr, R. T. LaLumiere, P. W. Kalivas and J. F. McGinty, *J. Neurosci.*, 2009, **29**, 3715–3719.
60. L. E. O'Dell, A. J. Roberts, R. T. Smith and G. F. Koob, *Alcohol Clin. Exp. Res.*, 2004, **28**, 1676–1682.
61. G. R. Valdez, A. J. Roberts, K. Chan, H. Davis, M. Brennan, E. P. Zorrilla and G. F. Koob, *Alcohol Clin. Exp. Res.*, 2002, **26**, 1494–1501.
62. L. A. Knackstedt, R. I. Melendez and P. W. Kalivas, *Biol. Psychiatry*, 2010, **67**, 81–84.
63. E. J. Bartlett, J. D. Brodie, P. Simkowitz, S. L. Dewey, H. Rusinek, N. D. Volkow, A. P. Wolf, G. Smith, A. Wolkin and R. Cancro, *Psychiatry Res.*, 1996, **60**, 91–99.
64. L. A. Knackstedt, K. Moussawi, R. Lalumiere, M. Schwendt, M. Klugmann and P. W. Kalivas, *J. Neurosci.*, 2010, **30**, 7984–7992.

65. D. A. Baker, K. McFarland, R. W. Lake, H. Shen, X. C. Tang, S. Toda and P. W. Kalivas, *Nat. Neurosci.*, 2003, **6**, 743–749.
66. K. Moussawi, A. Pacchioni, M. Moran, M. F. Olive, J. T. Gass, A. Lavin and P. W. Kalivas, *Nat. Neurosci.*, 2009, **12**, 182–189.
67. L. A. Knackstedt, S. LaRowe, P. Mardikian, R. Malcolm, H. Upadhyaya, S. Hedden, A. Markou and P. W. Kalivas, *Biol. Psychiatry*, 2009, **65**, 841–845.
68. S. D. LaRowe, P. Mardikian, R. Malcolm, H. Myrick, P. Kalivas, K. McFarland, M. Saladin, A. McRae and K. Brady, *Am. J. Addict.*, 2006, **15**, 105–110.
69. Z. X. Xi, S. Ramamoorthy, D. A. Baker, H. Shen, D. J. Samuvel and P. W. Kalivas, *J. Pharmacol. Exp. Ther.*, 2002, **303**, 608–615.
70. J. Peters and P. W. Kalivas, *Psychopharmacology (Berl.)*, 2006, **186**, 143–149.
71. Z. X. Xi, D. A. Baker, H. Shen, D. S. Carson and P. W. Kalivas, *J. Pharmacol. Exp. Ther.*, 2002, **300**, 162–171.
72. V. Kumaresan, M. Yuan, J. Yee, K. R. Famous, S. M. Anderson, H. D. Schmidt and R. C. Pierce, *Behav. Brain Res.*, 2009, **202**, 238–244.
73. P. J. Kenny, N. E. Paterson, B. Boutrel, S. Semenova, A. A. Harrison, F. Gasparini, G. F. Koob, P. D. Skoubis and A. Markou, *Ann. NY Acad. Sci.*, 2003, **1003**, 415–418.
74. P. Backstrom and P. Hyytia, *Neuropsychopharmacology*, 2006, **31**, 778–786.
75. H. S. Kalluri, A. K. Mehta and M. K. Ticku, *Brain Res. Mol. Brain Res.*, 1998, **58**, 221–224.
76. L. Trevisan, L. W. Fitzgerald, N. Brose, G. P. Gasic, S. F. Heinemann, R. S. Duman and E. J. Nestler, *J. Neurochem.*, 1994, **62**, 1635–1638.
77. J. T. Gass and M. F. Olive, *Biochem. Pharmacol.*, 2008, **75**, 218–265.
78. J. Besheer, J. J. Grondin, R. Cannady, A. C. Sharko, S. Faccidomo and C. W. Hodge, *Biol. Psychiatry*, 2010, **67**, 812–822.
79. P. Backstrom and P. Hyytia, *Eur. J. Pharmacol.*, 2005, **528**, 110–118.
80. M. F. Olive and H. C. Becker, *Alcohol*, 2008, **42**, 191–197.
81. M. A. Tanchuck, N. Yoneyama, M. M. Ford, A. M. Fretwell and D. A. Finn, *Alcohol*, 2011, **45**, 33–44.
82. M. L. Zeise, S. Kasparow, M. Capogna and W. Zieglgansberger, *Prog. Clin. Biol. Res.*, 1990, **351**, 237–242.
83. R. L. Popp and D. M. Lovinger, *Eur. J. Pharmacol.*, 2000, **394**, 221–231.
84. P. De Witte, J. Littleton, P. Parot and G. Koob, *CNS Drugs*, 2005, **19**, 517–537.
85. M. Simonetta Moreau, C. Cauhepe, J. P. Magues and J. M. Senard, *Br. J. Dermatol.*, 2003, **149**, 1041–1045.
86. I. P. Stolerman and M. J. Jarvis, *Psychopharmacology (Berl.)*, 1995, **117**, 2–10; discussion 14–20.
87. Stolerman and M. J. Jarvis, *Psychopharmacology*, 1995, **117**, 2–10.
88. W. A. Corrigall and K. M. Coen, *Psychopharmacology*, 1989, **99**, 473–478.
89. E. C. Donny, A. R. Caggiula, S. Knopf and C. Brown, *Psychopharmacology (Berl.)*, 1995, **122**, 390–394.

90. S. R. Goldberg, R. D. Spealman and D. M. Goldberg, *Science*, 1981, **214**, 573–575.
91. A. S. Rauhut, N. Neugebauer, L. P. Dwoskin and M. T. Bardo, *Psychopharmacology (Berl.)*, 2003, **169**, 1–9.
92. B. Le Foll, C. Wertheim and S. R. Goldberg, *PLoS One*, 2007, **2**, e230.
93. M. Sofuoglu, S. Yoo, K. P. Hill and M. Mooney, *Neuropsychopharmacology*, 2008, **33**, 715–720.
94. H. D. Mansvelder, J. R. Keath and D. S. McGehee, *Neuron*, 2002, **33**, 905–919.
95. P. Calabresi, M. G. Lacey and R. A. North, *Br. J. Pharmacol.*, 1989, **98**, 135–140.
96. W. A. Corrigall, K. M. Coen and K. L. Adamson, *Brain Res.*, 1994, **653**, 278–284.
97. W. A. Corrigall, K. B. Franklin, K. M. Coen and P. B. Clarke, *Psychopharmacology*, 1992, **107**, 285–289.
98. F. E. Pontieri, G. Tanda, F. Orzi and G. Di Chiara, *Nature*, 1996, **382**, 255–257.
99. M. R. Picciotto and W. A. Corrigall, *J. Neurosci.*, 2002, **22**, 3338–3341.
100. C. Silagy, T. Lancaster, L. Stead, D. Mant and G. Fowler, *Cochrane Database Syst. Rev.*, 2004, CD000146.
101. T. Baska, S. Straka, M. Baskova and R. Mad'ar, *Expert Opin. Pharmacother.*, 2004, **5**, 487–491.
102. H. J. Aubin, A. Bobak, J. R. Britton, C. Oncken, C. B. Billing, Jr, J. Gong, K. E. Williams and K. R. Reeves, *Thorax*, 2008, **63**, 717–724.
103. J. A. Stapleton, L. Watson, L. I. Spirling, R. Smith, A. Milbrandt, M. Ratcliffe and G. Sutherland, *Addiction*, 2008, **103**, 146–154.
104. P. J. Kenny, F. Gasparini and A. Markou, *J. Pharmacol. Exp. Ther.*, 2003, **306**, 1068–1076.
105. D. R. Helton, J. P. Tizzano, J. A. Monn, D. D. Schoepp and M. J. Kallman, *Neuropharmacology*, 1997, **36**, 1511–1516.
106. A. Madayag, D. Lobner, K. S. Kau, J. R. Mantsch, O. Abdulhameed, M. Hearing, M. D. Grier and D. A. Baker, *J. Neurosci.*, 2007, **27**, 13968–13976.
107. M. M. Moran, K. McFarland, R. I. Melendez, P. W. Kalivas and J. K. Seamans, *J. Neurosci.*, 2005, **25**, 6389–6393.
108. H. W. Shen, S. Toda, K. Moussawi, A. Bouknight, D. S. Zahm and P. W. Kalivas, *J. Neurosci.*, 2009, **29**, 2876–2884.
109. H. J. Groenewegen, C. I. Wright, A. V. Beijer and P. Voorn, *Ann. NY Acad. Sci.*, 1999, **877**, 49–63.
110. A. E. Kelley, *Ann. NY Acad. Sci.*, 1999, **877**, 71–90.
111. R. T. LaLumiere and P. W. Kalivas, *J. Neurosci.*, 2008, **28**, 3170–3177.
112. J. Le Merrer, J. A. Becker, K. Befort and B. L. Kieffer, *Physiol. Rev.*, 2009, **89**, 1379–1412.
113. S. D. Mague, A. M. Pliakas, M. S. Todtenkopf, H. C. Tomasiewicz, Y. Zhang, W. C. Stevens, Jr, R. M. Jones, P. S. Portoghesi and W. A. Carlezon, Jr, *J. Pharmacol. Exp. Ther.*, 2003, **305**, 323–330.

114. C. Chavkin, *Neuropsychopharmacology*, 2011, **36**, 369–370.
115. H. Kemppainen, N. Raivio, V. Suo-Yrjo and K. Kiianmaa, *Alcohol Clin. Exp. Res.*, 2012, **36**(2), 286.
116. A. J. Roberts, L. H. Gold, I. Polis, J. S. McDonald, D. Filliol, B. L. Kieffer and G. F. Koob, *Alcohol Clin. Exp. Res.*, 2001, **25**, 1249–1256.
117. C. K. Nielsen, J. A. Simms, J. J. Bito-Onon, R. Li, S. Ananthan and S. E. Bartlett, *Addict. Biol.*, 2012, **17**(2), 224.
118. S. C. Lapham and G. P. McMillan, *J. Addict. Med.*, 2011, **5**, 163–169.
119. W. C. Bryson, J. McConnell, T. Krothuis and D. McCarty, *Am. J. Manag. Care*, 2011, **17**(8), S222–S234.
120. J. C. Garbutt, H. R. Kranzler, S. S. O'Malley, D. R. Gastfriend, H. M. Pettinati, B. L. Silverman, J. W. Loewy and E. W. Ehrich, *JAM*, 2005, **293**, 1617–1625.
121. F. Berrendero, B. L. Kieffer and R. Maldonado, *J. Neurosci.*, 2002, **22**, 10935–10940.
122. C. L. Walters, J. N. Cleck, Y. C. Kuo and J. A. Blendy, *Neuron*, 2005, **46**, 933–943.
123. N. Ismayilova and M. Shoaib, *Psychopharmacology (Berl.)*, 2010, **210**, 211–220.
124. A. C. King and P. J. Meyer, *Pharmacol. Biochem. Behav.*, 2000, **66**, 563–572.
125. C. P. O'Brien and A. T. McLellan, *Lancet*, 1996, **347**, 237–240.
126. N. K. Mello, J. B. Kamien, J. H. Mendelson and S. E. Lukas, *NIDA Res. Monogr*, 1990, **105**, 617–618.
127. M. E. Carroll, S. T. Lac, M. J. Walker, R. Kragh and T. Newman, *J. Pharmacol. Exp. Ther.*, 1986, **238**, 1–7.
128. T. Kosten, D. G. Silverman, J. Fleming, T. A. Kosten, F. H. Gawin, M. Compton, P. Jatlow and R. Byck, *Biol. Psychiatry*, 1992, **32**, 543–548.
129. S. L. Walsh, J. T. Sullivan, K. L. Preston, J. E. Garner and G. E. Bigelow, *J. Pharmacol. Exp. Ther.*, 1996, **279**, 524–538.
130. J. M. Schmitz, A. L. Stotts, H. M. Rhoades and J. Grabowski, *Addict. Behav.*, 2001, **26**, 167–180.
131. M. J. Kreek, L. Borg, E. Ducat and B. Ray, *J. Addict. Dis.*, 2010, **29**, 200–216.
132. C. T. Chiu, T. Ma and I. K. Ho, *Brain Res. Bull.*, 2005, **67**, 100–109.
133. K. Anggadiredja, K. Sakimura, T. Hiranita and T. Yamamoto, *Brain Res.*, 2004, **1021**, 272–276.
134. J. E. Grant, B. L. Odlaug and S. W. Kim, *Eur. Neuropsychopharmacol.*, 2010, **20**, 823–828.
135. N. Jayaram-Lindstrom, A. Hammarberg, O. Beck and J. Franck, *Am. J. Psychiatry*, 2008, **165**, 1442–1448.
136. N. Jayaram-Lindstrom, M. Konstenius, S. Eksborg, O. Beck, A. Hammarberg and J. Franck, *Neuropsychopharmacology*, 2008, **33**, 1856–1863.
137. S. L. Borgland, S. A. Taha, F. Sarti, H. L. Fields and A. Bonci, *Neuron*, 2006, **49**, 589–601.
138. R. J. Smith, R. E. See and G. Aston-Jones, *Eur. J. Neurosci.*, 2009, **30**, 493–503.



139. S. J. Ward, M. Rosenberg, L. A. Dykstra and E. A. Walker, *Drug Alcohol Depend.*, 2009, **105**, 248–255.
140. J. S. Rodriguez, S. Y. Bocktor, L. C. Flores, C. F. Phelix and J. L. Martinez, Jr, *Neurosci. Lett.*, 2011, **489**, 187–191.
141. A. Elkashef, F. Vocci, G. Hanson, J. White, W. Wickes and J. Tiitonen, *Subst. Abuse*, 2008, **29**, 31–49.
142. S. M. Li, B. L. Campbell and J. L. Katz, *J. Pharmacol. Exp. Ther.*, 2006, **317**, 1088–1096.
143. T. S. Bondareva, R. Young and R. A. Glennon, *Pharmacol. Biochem. Behav.*, 2002, **74**, 157–162.
144. J. T. Hays, R. D. Hurt, N. A. Rigotti, R. Niaura, D. Gonzales, M. J. Durcan, D. P. Sachs, T. D. Wolter, A. S. Buist, J. A. Johnston and J. D. White, *Ann. Intern. Med.*, 2001, **135**, 423–433.
145. M. Quaak, C. P. van Schayck, D. S. Postma, E. J. Wagena and F. J. van Schooten, *Addiction*, 2012, **107**(1), 178.
146. A. M. Elkashef, R. A. Rawson, A. L. Anderson, S. H. Li, T. Holmes, E. V. Smith, N. Chiang, R. Kahn, F. Vocci, W. Ling, V. J. Pearce, M. McCann, J. Campbell, C. Gorodetzky, W. Haning, B. Carlton, J. Mawhinney and D. Weis, *Neuropsychopharmacology*, 2008, **33**, 1162–1170.
147. C. M. Reichel, J. E. Murray, K. M. Grant and R. A. Bevins, *Drug Alcohol Depend.*, 2009, **100**, 54–62.
148. B. M. Kinsey, D. C. Jackson and F. M. Orson, *Immunol. Cell Biol.*, 2009, **87**, 309–314.
149. J. J. Escobar-Chavez, C. L. Dominguez-Delgado and I. M. Rodriguez-Cruz, *Drug Des. Devel. Ther.*, 2011, **5**, 211–224.
150. B. A. Martell, F. M. Orson, J. Poling, E. Mitchell, R. D. Rossen, T. Gardner and T. R. Kosten, *Arch. Gen. Psychiatry*, 2009, **66**, 1116–1123.
151. J. E. Sturgess, T. P. George, J. L. Kennedy, A. Heinz and D. J. Muller, *Addict. Biol.*, 2011, **16**, 357–376.
152. F. Ducci and D. Goldman, *Addiction*, 2008, **103**, 1414–1428.
153. J. de Leon, *Neuropsychopharmacology*, 2009, **34**, 159–172.
154. H. Jornvall, J. O. Hoog, B. Persson and X. Pares, *Pharmacology*, 2000, **61**, 184–191.
155. H. R. Thomasson, H. J. Edenberg, D. W. Crabb, X. L. Mai, R. E. Jerome, T. K. Li, S. P. Wang, Y. T. Lin, R. B. Lu and S. J. Yin, *Am. J. Hum. Genet.*, 1991, **48**, 677–681.
156. G. Rubio, G. Ponce, R. Rodriguez-Jimenez, M. A. Jimenez-Arriero, J. Hoenicka and T. Palomo, *Alcohol Alcohol.*, 2005, **40**, 227–233.
157. A. C. King, J. R. Volpicelli, A. Frazer and C. P. O'Brien, *Psychopharmacology (Berl.)*, 1997, **129**, 15–22.
158. S. Krishnan-Sarin, J. H. Krystal, J. Shi, B. Pittman and S. S. O'Malley, *Biol. Psychiatry*, 2007, **62**, 694–697.
159. J. F. Rogers, A. N. Nafziger and J. S. Bertino, Jr, *Am. J. Med.*, 2002, **113**, 746–750.
160. G. Lurcott, *Anesth. Prog.*, 1998, **45**, 154–156.



161. Y. Gasche, Y. Daali, M. Fathi, A. Chiappe, S. Cottini, P. Dayer and J. Desmeules, *N. Engl. J. Med.*, 2004, **351**, 2827–2831.
162. J. Lotsch, C. Skarke, J. Liefhold and G. Geisslinger, *Clin. Pharmacokin.*, 2004, **43**, 983–1013.
163. M. Eichelbaum and A. S. Gross, *Pharmacol. Ther.*, 1990, **46**, 377–394.
164. D. W. Oslin, W. Berrettini, H. R. Kranzler, H. Pettinati, J. Gelernter, J. R. Volpicelli and C. P. O'Brien, *Neuropsychopharmacology*, 2003, **28**, 1546–1552.

## CHAPTER 15

# *Emerging Research towards the Understanding and Treatment of Autism*

PAUL WANG, REBECCA HAMMOND, FRISO POSTMA  
AND AILEEN HEALY\*

Seaside Therapeutics, 840 Memorial Drive, Cambridge, MA 02155, USA

\*Email: [ahealy@seasidetherapeutics.com](mailto:ahealy@seasidetherapeutics.com)

## 15.1 Autism and Autism Spectrum Disorders

The modern conceptualization of autism dates from the independent reports by Kanner and by Asperger, in the 1940s.<sup>1</sup> While the subjects described by Kanner and by Asperger differed markedly in their general cognitive abilities, they all shared the common symptom of being socially and emotionally isolated from other people, and even from close family members. In Kanner's words, individuals with autism are characterized by their "inability to relate . . . to people and situations from the beginning of life".<sup>2</sup> Kanner coined the term "autism" from the root "auto-," to reflect the fact that his patients seemed to be isolated unto themselves.

According to the most widely used diagnostic nosologies today (the DSM-IV<sup>3</sup> and the ICD-10<sup>4</sup>), autism is defined by symptoms in three domains: social skills, communication and repetitive behaviours and restricted interests. "Insistence on sameness", which refers to a pathological desire for routines in daily activities (*e.g.* the schedule of daily activities, or the menu for dinner each night) or

for a consistent physical environment (*e.g.* the arrangement of furniture in a room, or the clothing that a family member wears), is one of the symptoms within the domain of repetitive behaviours and restricted interests. Symptoms in each of the three domains may vary in severity, and the presence of symptoms in all three domains, with sufficient severity, results in the full DSM-IV diagnosis of “autistic disorder”. Patients with symptoms of lesser severity, or without significant symptoms in one or two domains, may be diagnosed with one of the other “autism spectrum disorders” (ASD; also known as “pervasive developmental disorders”). For example, patients are diagnosed with Asperger syndrome if they have no severe, early impairments in language development, but have all other symptoms of autistic disorder. Still other patients with symptoms of lesser severity may be diagnosed with “pervasive developmental disorder – not otherwise specified” (PDD-NOS).

The specific DSM-IV criteria for autistic disorder are shown in Table 15.1. Research over the last decade suggests that the symptoms from the first two domains, social skills and communication, are closely related to each other. It is anticipated that these two domains will be unified in the diagnostic criteria of the upcoming fifth edition of the DSM,<sup>5</sup> with repetitive behaviours and restricted interests remaining a separate domain of symptoms. The DSM-V also is anticipated to discard the diagnostic category of “Asperger syndrome”, because of arguments that it is theoretically indistinguishable from autistic disorder itself, and that current use of the Asperger diagnosis has become indiscriminately unfaithful to the criteria specified in DSM-IV and ICD-10.

While the DSM-IV and ICD-10 criteria for ASD are very closely aligned, two other diagnostic instruments have become the gold standard for research purposes, and increasingly for clinical care as well. In clinical research on ASD, the Autism Diagnostic Interview – Revised (ADI-R) and the Autism Diagnostic Observation Scale (ADOS) are typically used together as confirmation for DSM-IV diagnoses.<sup>6</sup> The ADI-R diagnostic classification is based on an interview with the patient’s parent, while the ADOS classification is based on direct observation of the patient in structured play situations. Of note, both the ADI-R and ADOS require rigorous training and certification for those administering these instruments.

Individuals with ASD often show other behavioural symptoms that are not part of the diagnostic criteria, but can greatly complicate their management.<sup>7</sup> These symptoms are referred to as secondary or associated symptoms of ASD, and include irritable and aggressive behaviours, self-injury, hypersensitivity to visual, auditory, tactile or other sensory stimuli, apparent inattention and sleep difficulties. In many cases, the origin of irritable, aggressive or self-injurious behaviours is believed to be anxiety or other discontent over externally imposed limitations in the ability of a patient to engage in their desired repetitive and restricted behaviours. Limitations in communicative behaviour, it is argued, prevent the patient from expressing their objections through other means. Some also believe that the core symptom of repetitive behaviours can represent an attempt at self-soothing in the face of anxiogenic stimuli.

**Table 15.1** Diagnostic criteria for autistic disorder.<sup>3</sup>

- 
- A. Six or more items from (1), (2) and (3), with at least two from (1), and one each from (2) and (3):
- (1) Qualitative impairment in social interaction, as manifested by at least two of the following:
    - (a) marked impairment in the use of multiple non-verbal behaviours such as eye-to-eye gaze, facial expression, body postures and gestures to regulate social interaction
    - (b) failure to develop peer relationships appropriate to developmental level
    - (c) a lack of spontaneous seeking to share enjoyment, interests or achievements with other people (*e.g.* by a lack of showing, bringing or pointing out objects of interest)
    - (d) lack of social or emotional reciprocity
  - (2) Qualitative impairments in communication as manifested by at least one of the following:
    - (a) delay in, or total lack of, the development of spoken language (not accompanied by an attempt to compensate through alternative modes of communication such as gesture or mime)
    - (b) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others
    - (c) stereotyped and repetitive use of language or idiosyncratic language
    - (d) lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level
  - (3) Restricted, repetitive and stereotyped patterns of behaviour, interests and activities, as manifested by at least one of the following:
    - (a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus
    - (b) apparently inflexible adherence to specific, non-functional routines or rituals
    - (c) stereotyped and repetitive motor manners (*e.g.* hand or finger flapping or twisting, or complex whole-body movements)
    - (d) persistent preoccupation with parts of objects
- B. Delays or abnormal functioning in at least one of the following areas, with onset prior to age 3 years:
- (1) Social interaction
  - (2) Language as used in social communication, or
  - (3) Symbolic or imaginative play
- C. The disturbance is not better accounted for by Rett's Disorder or Childhood Disintegrative Disorder.
- 

### 15.1.1 Prevalence of ASD

The prevalence of ASD in children is now commonly quoted to be around 1%, on the basis of epidemiological studies in the United States, coordinated by the Centers for Disease Control and Prevention (CDC). One study analyzed data from a telephone survey on over 90,000 children, ranging from 3 to 17 years in age. One in 91 (1.1%) of these children were reported by their parents to have an ASD, as corroborated at least once by a health care provider.<sup>8</sup> Another recent study analyzed data from a total population of 337,093 children at 8 years of age from 14 surveillance sites. One in 88 (1.1%) of these children were confirmed by clinician review of their records to have an ASD.<sup>9</sup> The prevalence estimates of 1:88 and 1:91 are much higher than reported in the older literature.<sup>10</sup>

One large factor in the current high prevalence of ASD diagnoses is the increase in awareness of autism that has occurred among parents, physicians and educators. Strong evidence also exists for a shift in diagnostic practices, such that the prevalence of “mental retardation” (also referred to as “intellectual disability” (ID) or in Britain as “severe learning disability”) has decreased markedly as the prevalence of ASD has increased.<sup>11</sup> Furthermore, one recent report finds no recent increase in prevalence. Using direct evaluation of a random sample of over 7,000 adults in England, the prevalence of ASD was found to be about 1.0%, matching the prevalence in children, and showing no age-related trend.<sup>12</sup> Yet unverified are the population-based screening data from a single city in South Korea, which estimated the prevalence of ASD to be 1:38, which is obviously higher than found in the two CDC studies conducted in the US.<sup>13</sup>

Arguments that the prevalence of ASD is increasing are often accompanied by assertions that there is an environmental agent responsible for the putative epidemic. Most eagerly designated as potential environmental culprits are childhood vaccines, and the mercury that was used as a preservative in some vaccines. In fact, there are no rigorous data demonstrating any association between these agents and ASD, and several studies have found that the incidence of autism did not increase when widespread vaccination practices were introduced, and did not decrease when the use of mercury-based preservatives was discontinued.<sup>14</sup>

## **15.2 Etiology of ASD (Defining the Molecular and Neuropathophysiology)**

### **15.2.1 Heritable Risk Factors**

The clinical and genetic heterogeneities of ASD have been a major challenge in defining etiology. The identification of chromosomal abnormalities, copy number variations, single-gene mutations and metabolic disorders that are causative or correlated with ASD number over 100; however, they account for only a minority of ASD cases.<sup>15,16</sup> The critical question remains: what causes autism? Development of the higher order functions misregulated in ASD, communication, behaviour and social interactions, rely upon proper neuronal development and the synaptic connections between neurons. The evidence to date suggests that multiple etiologies at the genetic, epi-genetic and environmental levels, working alone or in combination, converge on molecular pathways regulating neuronal development and synaptogenesis to cause autism.

Human brain development begins during the first weeks following conception and continues through early childhood. Highly conserved, well-defined signalling pathways regulate neuronal differentiation and migration, initial processes in brain development. Axonal outgrowth and guidance, dendritic branching and the formation of synapses between axons and dendrites begin *in utero* and continue into early post-natal development. Experiences in the first years of life further shape synaptogenesis, pruning of the dendritic arbor and

activity-dependent synaptic plasticity. At this time the cellular mechanisms necessary for proper synapse function are largely defined, including cell–cell and cell–matrix interactions, receptor-mediated signal transduction and organization of neuronal cyto-architecture. As experiences are shaping brain development, concerns about behavioural development first arise, often as missed early milestones, with an autism diagnosis usually not confirmed until approximately 3 years of age. The convergence hypothesis of autism suggests it is the improper development of this intricate biological network – connecting sensorimotor functions, behaviour and cognition – that leads to autism. In accordance with this hypothesis, perturbations in essential components of these systems are being revealed as causative agents in ASD.

## 15.2.2 Chromosomal Rearrangements

Significant effort has focused on the genetic causes of autism. Concordance among monozygotic twins and the higher recurrence risk to siblings with ASD provides strong evidence for a familial effect.<sup>17,18</sup> Chromosomal abnormalities resulting from duplications, deletions or rearrangements are known causes of ASD; however, many of these types of chromosomal abnormalities are syndromic and autism is just one of many developmental phenotypes expressed. These prevalent abnormalities include alterations at 2q37, 15q11-q13, 16p11.2, 22q11.2 and 22q13.3.<sup>19–22</sup> The most frequent of these is the duplication at 15q11-q13,<sup>23</sup> which is estimated to occur in 0.5–3% of ASD cases.<sup>24</sup> In addition to an increased susceptibility to ASD, 15q11-q13 duplications are also associated with increased risk of ID, seizures and schizophrenia.<sup>25</sup> Parent-specific inheritance of 15q11-q13 duplications also affects risk; maternal inheritance poses a significant risk for developing ASD while paternal inheritance of 15q11-q13 duplications shows a reduced penetrance for ASD.<sup>23,26</sup>

The parent-specific inheritance is explained by the discovery that the 15q11-q13 locus is one of several gene clusters found throughout the genome that is regulated by imprinting. Imprinting is a differential mark that occurs in the parental germ-line and is maintained in the somatic cells of the offspring. Imprinting centres (IC) regulate the marking of gene clusters to confer parent-of-origin-specific gene expression. This can occur through DNA methylation, histone modification, non-coding RNA gene inactivation, chromatin interactions (called looping) and binding of insulator elements to create active and inactive chromatin domains.<sup>27</sup> Imprinting tightly controls gene expression throughout growth and neurologic development, therefore mutations at imprinting centres in these clustered sites have a significant impact on global physiological development.<sup>28</sup> The IC in the 15q11-q13 loci is a bipartite regulator, conversely imprinting paternal- or maternal-inherited genes. Deletions at 15q11-q13 exemplify this, with parental inheritance governing two distinct neurodevelopmental disorders: Prader–Willi Syndrome (PWS) resulting from paternal inheritance and Angelman Syndrome (AS) resulting from maternal inheritance.

PWS is characterized by hypotonia from birth, short stature, global developmental delays and hyperphagia. Cognitive, social and motor deficits are also typical of PWS, with a diagnosis of autism in approximately 25% of cases. The majority (70%) of PWS cases are caused by a deletion of the entire 15q11.2-q13.1 region. Another 25% of cases are caused by maternal uniparental disomy, where both copies of chromosome 15 are inherited from the mother (with imprinting silencing the paternal genes). Less than 5% of cases result from a defect in the imprinting centre, which also negatively affects expression of the paternal genes. Although the genes in this region are mapped, the single or multiple genes causing PWS have not been identified.

Angelman Syndrome (AS) is characterized by developmental delays, impaired language or complete absence of speech, seizures, ID, motor deficits and autism. Maternal deletions of chromosome 15q11-q13 occur in about 70% of cases, paternal uniparental disomy (2%), imprinting defects (2%) and ubiquitin protein ligase E3A (*UBE3A*) deletion or point mutations (5–10%) comprise the remainder, while a small percentage of causes remain undefined. Analyses of point mutations in AS patients revealed that *UBE3A* is the gene causing AS.<sup>29</sup> In brain, *UBE3A* shows maternal-allele specific expression,<sup>30</sup> with silencing of the paternal allele by a *UBE3A* antisense strand expressed only from paternally inherited alleles.<sup>31</sup> Because *UBE3A* expression is imprinted only in brain cells, defining the brain-specific function of *UBE3A* has revealed insights into autism susceptibility (see Section 15.2.4.2).

Another chromosomal rearrangement associated with cognitive impairment is the 22q13.3 micro-deletion syndrome (Phelan–McDermid syndrome). Similar to developmental disorders associated with other chromosomal rearrangements, 22q13.3 micro-deletion syndrome is characterized by global developmental delays, ID, absent or delayed speech and autistic behaviour. Examination of the three genes mapped to this region, *SHANK3*, *ACR* and *RABL2B*, suggests *SHANK3* is an autism susceptibility gene. Clinical studies show that a small subgroup of ASD patients exhibit mutations in the *SHANK3* gene and suggest abnormal gene dosage of *SHANK3* is linked to intellectual disabilities, and the autistic phenotypes of impaired social interactions and speech.<sup>32</sup> These observations led scientists to develop a *SHANK3*-deficient mouse.<sup>33,34</sup> *SHANK3* is a scaffold protein localized to the post-synaptic density of excitatory synapses, a dense lattice of proteins coupling membrane receptors to the actin cytoskeleton. Knockdown or over-expression of *SHANK3* in mouse neurons affects dendritic spine formation and synaptic function.<sup>33</sup> A more recent report of an isoform-specific, *SHANK3*-deficient mouse shows altered dendritic spine morphology, alterations in  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) trafficking and deficiencies in long-term potentiation (LTP). Social behaviour and cognitive performance were impaired in the *SHANK3*-deficient mice as compared with wild-type control mice, suggesting the cellular and physiologic alterations lead to the behavioural abnormalities.<sup>34</sup>

The chromosomal rearrangements described here lead to syndromic forms of autism. However, these prevalent chromosomal abnormalities provide insight into non-syndromic, idiopathic autism by defining sensitivity to gene dosage as



contributing to abnormal brain function. In addition, the mutated genes in AS and Phelan–McDermid syndrome affect synaptic mechanisms, suggesting there are common cellular functions that converge on specific brain function. Further investigations are warranted to identify and determine if any of these mutated genes provide novel druggable targets for autism.

### 15.2.3 Copy Number Variations

Copy number variations (CNVs) are micro-deletions and duplications of DNA segments found throughout the genome and are part of the normal repertoire of human genetic variation. Thousands of CNVs have been identified and hundreds are associated with ASD. Genome sequencing studies have shown that most nucleotide bases that vary among genomes reside in CNVs of at least 1 kilobase in size.<sup>35</sup> Early detection methods of large (several megabases) CNVs consisted of karyotyping and fluorescence *in situ* hybridization. These methods were used to identify most of the chromosomal abnormalities discussed in Section 15.2.2. Currently, CNVs are detected with increasing resolution using genome-wide micro-array technologies.<sup>35</sup> The prevalence of CNVs can be rare and unique within a family, or common and present in up to 1% of the population. When a CNV is present in two or more unrelated families, it is considered “recurrent”, a designation required to establish a causal link between a mutation and autism susceptibility.

The most frequent recurrent *de novo* CNVs occur at regions 16p11.2, 15q11.2-13.1, 7q11.23, 22q11.2 and the NRXN1 gene at 2p16.3, with 16p11.2 deletions and duplications<sup>36–39</sup> showing the strongest evidence for genome-wide recurrent association.<sup>40–43</sup> The elevated mutation rate at 16p11.2 results from micro-deletions and duplications caused by segmental duplications (recombination hotspots) flanking 16p11.2. The occurrence of the 16p11.2 CNVs among ASD probands is 1.1–1.2%, making it a common cytogenetic cause of autism with a frequency similar to maternal duplications of 15q11-13.

Several rare recurrent *de novo* CNVs are mapped and include 1q21, 7q11.23, 15q13.2-13.3, 16p13.2 and Cadherin13 (CDH13) at 16q23.3.<sup>42,44</sup> A *de novo* CNV at 20p13 involves 27 genes including the oxytocin gene.<sup>36</sup> Oxytocin is a hormone synthesized in the hypothalamus that regulates the development of pro-social, attachment, approach and stress behaviour. Compared to control subjects, plasma levels of oxytocin were found to be lower in autistic children whereas precursor oxytocin levels were higher.<sup>45,46</sup> In several studies, variants in the receptor for oxytocin (*OXTR*, at 3p25) were associated with autism.<sup>47–49</sup> Furthermore, mice deficient in oxytocin receptors show social recognition defects;<sup>50</sup> however, the neurochemical mechanism(s) remains undefined. Mapping the CNVs is a critical first step towards defining the causative genes for autism susceptibility.

The recent results of a number of genome-wide association studies (GWAS) examining CNVs provide a framework to define autism etiology. The “common disease-common variant” model of ASD posits common (allele frequencies > 1%) CNVs with modest individual effects interact and contribute to

the disorder in an additive or multiplicative manner. GWAS for *common* genetic risk factors identified several regions associated with ASD such as 5p15.1 between Cadherin10 (CDH10) and Cadherin9 (CDH9)<sup>51</sup> and 5p15.2 between Semaphorin-5A (SEMA5A) and Taste Receptor Type 2 (TAS2R1).<sup>37</sup> Results of a study from the Autism Genome Project Consortium identified 20p12.1 (*MACROD2*) as the most significant locus associated with ASD.<sup>52</sup> Despite these findings, no shared regions were identified between studies. Studies from the Simons Simplex Cohort<sup>42</sup> did identify multiple rare transmitted or *de novo* CNVs corresponding to these loci. However, the associated odds ratios reported in the GWAS for common genetic traits are small, which suggests common genomic variation accounts for only a small fraction of familial idiopathic ASD.

The “common disease-rare variant” model hypothesizes multiple, rare variants of high penetrance underlie disease susceptibility. In support of this model, large, rare *de novo* CNVs associated with ASD occur more often in families with one affected child (simplex) *versus* families with multiple affected siblings (multiplex).<sup>36</sup> *De novo* CNVs account for 5–11% of simplex autism cases.<sup>38,42,53,54</sup>

The list of CNVs associated with ASD contains hundreds of genes, but many cluster into molecular networks related to synapse development, axon targeting and neuron motility.<sup>55</sup> For example, the CNVs of 16p11.2 contain 25 genes in which 12 genes were mapped to a single genetic network with a function in cell–cell signalling and interactions. The pathways for three of the 25 genes (*DOC2A*, *MAPK3* and *ALDOA*) include post-synaptic density genes that have been hypothesized to underlie autism.<sup>39</sup> For example, mitogen-activated protein kinase 3 (*MAPK3*) is expressed in the developing and adult human brain and *MAPK3*-deficient mice display abnormal avoidance behaviour, hyperactivity, reduced long-term potentiation (LTP) and immune system abnormalities.<sup>56</sup> Continuing efforts to identify and understand which CNVs are associated with autism and define the responsible genes will advance our ability to identify targets for treatments.

## 15.2.4 Rare Mutations with Known Etiology

### 15.2.4.1 Adhesion Molecules

Neuronal cell–cell interactions that stabilize synapse formation are now understood to be critical for proper brain function. The neurexin-neurexin (NRXN-NLGN) complexes are trans-synaptic adhesion molecules, first described as cell recognition molecules<sup>57</sup> and now understood to be required organizing factors in synapse formation, differentiation and function.<sup>58,59</sup> There are four neuroligin genes that when expressed are inserted in the post-synaptic membrane and function as ligands for the neurexins. Three neurexin genes expressed as either alpha or beta isoforms are the pre-synaptic membrane receptors for neuroligins. Splice variants increase the complexity of receptor–ligand coupling. The initial descriptions linking neuroligins to autism were

reports of association with autism at Xq13-21 and Xp22.3 chromosomal deletions.<sup>60,61</sup> A subsequent study identified a single C to T transition in neuroligin-3 (*NLGN3*), resulting in the amino acid substitution mutation at Arg451Cys, found in siblings with ASD. In a second family, a frame-shift mutation at 1186insT causing a truncation mutation in *NLGN4* was identified.<sup>60,62</sup> *NLGN3* is located at Xq13-21. *NLGN4* lies within the Xp22.3 deletion interval. Heterologous expression of the Arg451Cys mutation revealed how the mutation affected *NLGN3* function.<sup>63</sup> The Arg451Cys polymorphism results in a local folding defect causing *NLGN3* to remain in the endoplasmic reticulum, thus preventing normal trafficking to the post-synaptic membrane and diminished ligation to  $\beta$ -NRXN.<sup>63</sup> In addition to mutations in *NLGN3* and *NLGN4* that predispose to autism, a heterozygous deletion of NRXN1 $\alpha$  is a susceptibility factor for autism and schizophrenia.<sup>64-67</sup>

The recent identification of additional polymorphisms in *NLGN4* resulted from sequencing the entire coding region including 1 kilobase of the 5' UTR. A single *de novo* 1-base pair substitution G>A located in the promoter region sequence 335 base pairs upstream from the transcription initiation site was identified in a boy with autism and profound ID.<sup>68</sup> Expression levels were altered over two-fold in peripheral blood cells from this patient, suggesting a gain-of-function mutation. Although this finding is not conclusive, it is important because it suggests that rare mutations affecting gene dosage are causative.<sup>69</sup>

Many additional examples of cell-adhesion molecules implicated in ASD and found associated with the synaptic membrane include members of the cadherin/protocadherin families, the contactins, contactin-associated proteins and the extra-cellular matrix protein, reelin. However, most of the mutations are rare variants and the core pathophysiologic mechanisms remain undefined. Moreover, cell-adhesion molecules are not druggable targets, further emphasizing the need to define the associated synaptic signalling pathways controlling synaptic structure and function.

### 15.2.4.2 Single Gene Disorders

The single gene disorders, Fragile X Syndrome (FXS), Tuberous sclerosis complex (TSC) and PTEN Hamartoma Tumour Syndrome (PHTS), each involve rare mutations that converge on mechanisms controlling neuronal protein synthesis. The high association of each of these single gene disorders with autism suggests defects in the pathways linking synaptic signal transduction to neuronal protein synthesis underlie autism susceptibility.

The transcriptional silencing of the single gene, Fragile X Mental Retardation 1 (*FMRI*), causes FXS. Approximately 18–33% of individuals with FXS are also diagnosed with autism, and the prevalence of FXS in autism is 2–4%,<sup>70,71</sup> making it one of the most common single-gene disorders associated with autism. FXS is characterized by cognitive and behavioural deficits including developmental delay, attention deficits and hyperactivity, impulsivity, aggression, abnormalities in language and communication, social anxiety and

stereotyped behaviours and interests.<sup>72,73</sup> The FXS mutation results from the expansion and methylation of a trinucleotide repeat in the 5' untranslated region (UTR) of the *FMRI* gene. The *FMRI* gene product, FMRP, is an RNA-binding protein that represses local protein synthesis through a mechanism of RNA interference.<sup>74–77</sup> In the absence of FMRP, hundreds of target mRNAs localized to dendritic spines are translated in excess,<sup>78</sup> and this mechanism is thought to underlie the core phenotypes in FXS. The neuro-pathophysiology underlying FXS was largely revealed through extensive studies of the *Fmr1*-deficient mouse.<sup>79</sup> The *Fmr1*-deficient mouse exhibits excessive hippocampal protein synthesis, exaggerated long-term depression (LTD) and increased dendritic spine density. These changes are thought to underlie the behavioural phenotype of these mice, which includes increased locomotor activity, excessive memory extinction and susceptibility to stimulus-induced seizures.<sup>79–81</sup>

TSC is caused by heterozygous mutations in the genes encoding either hamartin (TSC1) or tuberlin (TSC2)<sup>82</sup> and is characterized by hamartomas or tumours along with deficits that include cognitive impairment, epilepsy and a high prevalence of ASD (20–60%).<sup>83,84</sup> TSC1 and TSC2 form heterodimers allowing TSC2, which is a GTPase activating (GAP) protein, to catalyze the inactivation of Rheb and the subsequent inhibition of the mammalian target of rapamycin (mTOR) kinase. mTOR is assembled into two protein complexes, mTORC1 and mTORC2. mTORC1 controls translation through two kinase-dependent signalling cascades. First, mTORC1 activates cap-dependent translation initiation through 4EBP phosphorylation and subsequent de-repression of the cap-dependent initiation factor, eIF4E.<sup>85</sup> Second, mTORC1 phosphorylates the p70 ribosomal protein kinase, S6 (S6K), which stimulates synthesis of the ribosomal machinery required for translation. Loss-of-function mutations in TSC1/2 result in constitutive activation of mTOR.<sup>86–88</sup> The results of a recent study showed that mGluR-LTD is absent in hippocampal neurons deficient in *Tsc1*,<sup>89</sup> which is the converse of the phenotype observed in the *Fmr1*-deficient neurons. Because mGluR-LTD is protein-synthesis dependent, it is likely that altered synthesis of the ribosomal machinery in *Tsc1*-deficient mice negatively affects protein synthesis. *Tsc1* and *Fmr1* loss-of-function mutants reveal two distinct pathways affecting protein synthesis and synaptic plasticity that converge on pathologies with shared symptoms, namely cognitive impairment, seizures and autism.

Genetic mutations in the tumour suppressor gene, PTEN (phosphatase and tensin homologue deleted on chromosome ten), cause a familial cancer syndrome (Cowden syndrome) and related hamartoma syndromes associated with macrocephaly, seizures, ASD and ID.<sup>90,91</sup> PTEN is a lipid phosphatase and one of two negative regulators of phosphoinositide 3-kinase (PI3K). PI3K catalyzes the phosphorylation of the membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3). PTEN dephosphorylates PIP3, thereby terminating PI3K signal transduction.<sup>92</sup> Loss-of-function PTEN mutations result in constitutive activation of downstream mediators of the PI3K pathway, including AKT and mTOR. Constitutive

activation of the AKT/mTOR pathway in TSC and PTEN-related syndromes may contribute to macrocephaly. Macrocephaly occurs in approximately 20% of ASD patients and almost 5% of ASD patients with macrocephaly have been identified with germline mutations in PTEN.<sup>91,93</sup> *Pten*-deficient mice develop macrocephaly, and this phenotype can be corrected by treating mutant mice with the mTORC1 inhibitor, rapamycin.<sup>94</sup> Examination of cellular morphology in *Pten*-deficient mice revealed neuronal hypertrophy including increased dendritic size and axonal thickness.<sup>95</sup> mTORC1 regulates cell size by phosphorylating S6K and 4EBP1, which in turn promote protein synthesis. Therefore silencing mutations in PTEN may be directly linked to macrocephaly through translational control.

The hypothesis that autism susceptibility is increased by genetic mutations affecting synaptic translation is further supported by the link between neurofibromatosis and autism. ASD patients have a significantly increased risk of neurofibromatosis, suggesting a shared etiology. The cause of neurofibromatosis is loss-of-function mutations in neurofibromin (NF1), a tumour suppressor that mediates Ras signalling through the MAPK and PI3K pathways. NF1-deficiency results in the constitutive activation of Ras-dependent ERK and mTOR signalling.<sup>96</sup> Increased protein synthesis is a predicted consequence of up-regulated mTOR. Consistent with this, NF1-deficient astrocytes over-express proteins involved in ribosomal processing and assembly.<sup>97</sup>

In AS, 15q11-q13 chromosomal abnormalities resulting in monoallelic expression of *UBE3A* demonstrate the importance of this protein in brain development and provide insight into the causes of cognitive impairment. *UBE3A* is an enzyme that tags proteins for ubiquitination and degradation, and in neurons expression is enriched in the nucleus and dendritic spines *in vivo*.<sup>98</sup> *Ube3A*-deficient mice show impaired experience-dependent synaptic plasticity,<sup>99</sup> suggesting that ubiquitination and degradation of effectors at neuronal synapses is key to normal brain function. The evidence obtained from studying the *Ube3A*-deficient mouse identifies an alternative pathway by which synaptic proteins can accumulate, *i.e.* perturbations in protein turnover, and defines another point of convergence for the misregulation of synaptic function.

The results of recent studies identified synaptic proteins that are targets of *UBE3A* and defined the role of each as effector molecules in synapse formation and function. Ephexin 5 is one such effector molecule.<sup>100</sup> Ephexin 5, a RhoA guanine nucleotide exchange factor (GEF), that is part of the Eph-Ephrin signalling pathway,<sup>101</sup> suppresses the formation of excitatory synapses. Ephexin 5 expression *in vitro* is inversely correlated with the number of excitatory synapses.<sup>102</sup> Synapse formation occurs when Ephexin 5 expression is reduced *via* *Ube3A* activity. EphrinB activates EphB, a receptor tyrosine kinase, triggering an enzymatic-signalling cascade resulting in the phosphorylation of Ephexin5. Phosphorylated Ephexin 5 is then ubiquitinated by *Ube3A* and degraded, thereby removing a restriction point in synapse formation. In the absence of *Ube3A*, Ephexin 5 levels remain high and synapse number is decreased.

A second effector molecule identified from the same initial screen of Ube3A substrates is Arc,<sup>100</sup> an immediate early gene product acting downstream of signalling pathways predominantly found in glutamatergic neurons. Arc is proposed to play a vital role in neuronal homeostasis.<sup>103</sup> In response to glutamate-mediated synaptic activation, post-synaptic Arc expression increases and directly mediates AMPAR endocytosis. AMPARs are ionotropic glutamate receptors that mediate fast synaptic transmission. Thus Arc limits further neuronal excitation by removing AMPARs from the synaptic surface. *Ube3A* transcription is also induced following activation at excitatory synapses, but with slower induction kinetics than *Arc*. Ube3A regulates Arc levels through a temporally controlled binding, ubiquitination and degradation of Arc.<sup>100</sup> In the absence of Ube3A, increased Arc expression leads to continuous AMPAR endocytosis directly impairing synaptic transmission. Collectively, the results of these studies provide mechanistic insight into how deletion of the Ube3A gene can directly affect synapse formation and function and identify new areas for the development of therapeutic interventions.

Rett syndrome (RS) is caused by loss-of-function mutations in the gene encoding the methyl-CpG binding protein 2 (MECP2) and is characterized by a progressive loss of developmental milestones beginning around the first year and including the loss of verbal skills and purposeful hand movements, and seizures and ASD. Unlike the other rare single-gene disorders described that regulate protein synthesis, MECP2 regulates gene transcription. MECP2 was originally described as a global repressor of transcription acting through DNA methylation and silencing of target genes; however, recent evidence demonstrates MECP2 also activates gene transcription.<sup>104,105</sup> Because the number of MECP2 target genes is estimated in the hundreds, the altered transcription of MECP2 target genes presumably alters the composition of synaptic proteins, which in turn affects synaptic connectivity.

The recent studies focusing on synaptic regulation of protein synthesis in ASD support the convergence hypothesis of autism. This is based on the observation that the gene products affected in the single-gene disorders act as negative regulators of synaptic protein synthesis.<sup>106</sup> In addition to FXS, protein products of the genes mutated in TSC, NF1, AS and PHTS share in common a role in regulation of activity-dependent protein synthesis at neuronal synapses. Current studies are examining genetically engineered mouse models of the single-gene disorders associated with ASD to understand the effects of controlling altered neuronal protein synthesis, and the results of these studies will test the hypothesis of shared common processes increasing autism susceptibility.<sup>89,107–109</sup> By defining the neuropathophysiology of the single-gene disorders associated with autism, the development of therapeutics aimed at normalizing neuronal protein synthesis as a treatment for autism is now possible.<sup>106</sup>

The chromosomal rearrangements, CNVs and single-gene disorders described represent just some of the known (epi)genetic mutations causing ASD. A recurrent finding indicates that subtle alterations in gene dosage affecting synapse structure or function has profound effects on normal brain



development. FXS has previously been described as a “synapsopathy”;<sup>110</sup> the common mechanisms contributing to autism susceptibility suggest autism is also a disorder of the synapse. Consistent with this concept, a recent genome-wide expression analysis study comparing autistic and control brain RNA revealed discrete gene expression profile groupings correlated with disease status and these gene groupings are enriched in a neuronal network involved in synaptic function.<sup>111</sup>

### 15.2.5 Environmental Factors

The role environmental factors play in the etiology of ASD has not been systematically studied, and only a few studies suggest pre-natal exposure to environmental factors increases autism susceptibility. Teratogen exposure during pre-natal development is linked with autism, but most examples are from single case reports or select groups. In a single early report the incidence of autism was reported in 4 out of 100 patients affected by thalidomide embryopathy<sup>112</sup> and in a separate report, 5 patients with foetal valproate syndrome showed manifestations of autism.<sup>113</sup> Subsequent studies mainly focus on rodent models of altered behaviour following pre-natal exposure to teratogens. One such study is noteworthy: valproic acid administered to pregnant rats generates offspring with developmental delays, motor impairments and altered social behaviour. Although valproic acid is reported to affect expression of many genes, a recent report indicates that mRNA levels for NLGN3 are reduced in the offspring of valproic acid-treated animals compared to untreated controls, and the other NLGN and NRXN family members were not affected by drug treatment.<sup>114</sup> Alcohol exposure during pre-natal development is often cited as a possible risk factor for autism; however, a population-based prospective study in the Danish population examining >80,000 children, found no increased risk of autism from alcohol consumption during pregnancy.<sup>115</sup> Maternal exposure to selective serotonin reuptake inhibitors, during the first trimester, may increase autism susceptibility.<sup>116</sup> Advanced parental age has also been identified as a potential environmental risk for autism.<sup>117</sup> Finally, results from a recent study examining dizygotic and monozygotic twin susceptibility to autism suggest that the shared environment during development plays a significant role in autism susceptibility;<sup>118</sup> however, there was no attempt to identify potential environmental factors in this report. There is a clear need to utilize rigorous scientific methods to define the contribution of environmental factors to autism etiology and there have been recent calls to action from many of the foundations supporting autism research.

## 15.3 Current Treatments for ASD

Educational and behavioural therapies are the mainstay of treatment for the core, diagnostic symptoms of ASD.<sup>7</sup> It is generally held that earlier initiation of these treatments is associated with greater efficacy, but the evidence



for this claim is sparse. Given the nature of these approaches, it is impossible to conduct double-blind assessments of their efficacy. Even unblinded but controlled trials are rare, because it would be unethical to provide no treatment at all to children with ASD, and “wait-list control” groups typically receive widely varying interventions. Further complicating the assessment of treatment effects from these interventions is the fact that spontaneous improvement, at least to some degree, can occur in the symptoms of autism, over the course of years.

There are no drugs approved for the treatment of the core symptoms of ASD, though risperidone and aripiprazole are approved for treatment of the associated symptom of irritability (see below). Efforts to develop treatments of core symptoms have been few, as the pathophysiology of ASD was unknown until recently, and the condition was regarded as largely intractable. Families and physicians have, nonetheless, availed themselves of many off-label treatments in an attempt to manage either core symptoms of ASD, or associated symptoms of anxiety, irritability and sleep disturbance.<sup>119</sup> Commonly prescribed treatments include the antipsychotics, as discussed below, selective serotonin reuptake inhibitors (SSRIs) for anxiety and for repetitive behaviours, sedative-hypnotics, mood stabilizers and psychostimulants. None of the off-label treatments have a strong evidence base in ASD,<sup>120</sup> and meta-analyses on specific treatments (*e.g.* SSRIs) have not yielded support for efficacy.<sup>121</sup>

Risperidone and aripiprazole are labelled in the United States “for the treatment of irritability associated with autistic disorder” in patients 6–17 years of age (risperidone) or 5–16 years (aripiprazole).<sup>122</sup> Efficacy for both drugs was established in patients with full autistic disorder (*i.e.* patients with Asperger disorder or pervasive developmental disorder – not otherwise specified were not permitted to enroll) in 8-week trials using the Irritability subscale of the Aberrant Behaviour Checklist as the primary endpoint.<sup>123,124</sup> The approved labels for both state that dosage should be individualized for each patient, on the basis of tolerability and response, and the side-effect profiles for these medications appear to be very similar when prescribed to patients with autistic disorder as when prescribed for other approved indications.

Clinical experience with these and other antipsychotic medications demonstrates that they have clear utility in the treatment of irritability.<sup>125</sup> However, as Erickson *et al.* note, “neither risperidone or [sic] aripiprazole has been associated with improvement in social or communication impairment in youth with autism”.<sup>125</sup> Moreover, adverse events such as sedation, somnolence and fatigue are extremely common when these agents are administered to patients with ASD, and much higher than reported for placebo. The FDA-approved label for risperidone<sup>122</sup> reports a 63% incidence of somnolence and a 42% incidence of fatigue, both more than three times higher than on placebo. For aripiprazole,<sup>122</sup> the labelled incidences are lower, but are still more than five times higher than on placebo.

## 15.4 Emerging Treatments for ASD

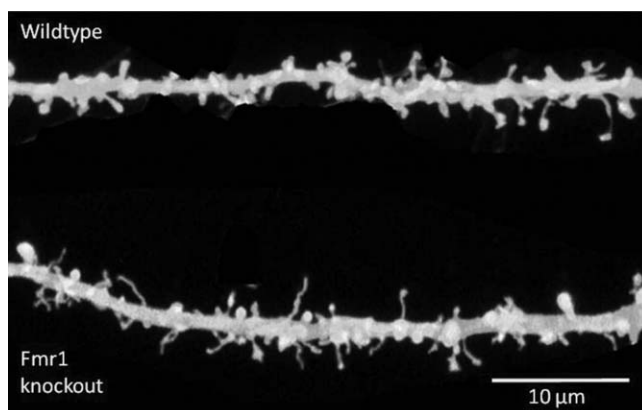
### 15.4.1 The mGluR Theory of FXS

The mGluR theory of FXS is founded in an understanding of the role group I metabotropic glutamate receptors (mGluR1 and mGluR5) play in experience-dependent synaptic plasticity, a bi-directional process. Glutamate mediates the majority of excitatory neurotransmission by activating ionotropic (*e.g.* AMPA, NMDA and kainate receptors) and metabotropic (G-protein coupled, mGluR) receptors. Post-synaptic glutamate receptor activation triggers the long-term changes in synaptic strength, *i.e.* long-term potentiation (LTP) and long-term depression (LTD), which are key mechanisms shaping learning and memory.<sup>126</sup> One type of LTD, mGluR-LTD, occurs when glutamate activates group I mGluRs, stimulating protein synthesis of pre-existing mRNA located in the post-synaptic dendrite.<sup>127,128</sup> One of the proteins rapidly synthesized in response to mGluR-LTD is FMRP and in the absence of FMRP mGluR-LTD is exaggerated.<sup>129</sup> This finding suggests that FMRP limits mGluR-LTD by inhibiting synaptic protein synthesis.<sup>130</sup> The mGluR theory of FXS is based on these findings<sup>130</sup> and can be simply described as controlled opposition between group I mGluRs and FMRP. In this model, the group I mGluRs are the “accelerator”, driving activity-dependent protein synthesis to stabilize LTD of synaptic strength, and FMRP is the “brake”, suppressing synaptic protein synthesis to limit LTD.<sup>130</sup> In the absence of FMRP, excessive synaptic protein synthesis downstream of mGluRs is the central pathogenic mechanism of FXS.

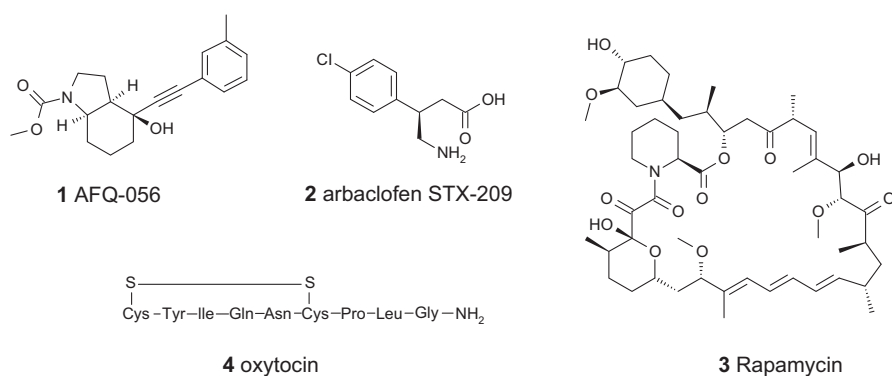
The mGluR theory of FXS predicts that reducing mGluR activity will correct the underlying neuropathophysiology and therefore correct the core symptoms of the disease. This hypothesis was tested using a genetic approach in a mouse model of FXS. *Fmr1*-deficient mice genetically engineered to express 50% of the wild-type level of mGluR5 showed correction of all neurologic phenotypes tested.<sup>131</sup> Reduction in mGluR5 activity rescued the excessive hippocampal protein synthesis, the exaggerated LTD and the increased dendritic spine density (see Figure 15.1). It is important to note that behavioural phenotypes were also corrected, such as the excessive memory extinction, the increased locomotor activity and the susceptibility to stimulus-induced seizures.<sup>131</sup> Complementary results have been obtained using a negative allosteric modulator (NAM) of mGluR5 in fragile X model mice, zebrafish and fruit flies.<sup>70</sup> Together, these studies suggest mGluR5 modulators may provide effective treatment by reducing cerebral protein synthesis downstream of mGluR5.

### 15.4.2 mGluR Negative Allosteric Modulators in FXS and ASD

The critical barrier for treating ASD and other developmental brain disorders has been a lack of understanding the core pathophysiologic mechanisms that lead to altered brain function. The prevalence of FXS in autism is 2–4%,<sup>70,71</sup> making it one of the most common single-gene disorders associated with



**Figure 15.1** FMRP is required for normal development of dendritic spines.



**Figure 15.2** Chemical structures of compounds in clinical development.

autism. Based on this overlap in phenotypes, there may also be overlap in the neural mechanisms underlying the two disorders. Understanding how the single-gene alteration in FXS results in autistic-like behaviours may provide valuable insights into potential targets for research on idiopathic autism.<sup>70</sup>

The mGluR5 theory of FXS is currently being tested in FXS patients. Novartis Pharma AG reported positive outcomes using the mGluR5 NAM, AFQ056,<sup>132</sup> (see Figure 15.2) in a randomized double-blind, placebo-controlled trial in FXS patients. Although no significant effects of treatment were detected on the primary outcome measure (the ABC-C score measured on day 19 or 20 of treatment), an exploratory analysis pointed to a statistically significant ( $P < 0.001$ ) improvement in the ABC-C score for the subset of seven patients who had full methylation of the *FMR1* promoter and no detectable *FMR1* mRNA. Based on these results, Novartis initiated a placebo-controlled 12-week

treatment study with AFQ056 in November 2010 to further assess safety and efficacy in 160 adult FXS patients, 18–45 years of age. The primary outcome measure is change from baseline in behavioural symptoms of FXS using the ABC-C Total score. Hoffmann La-Roche is recruiting adult FXS patients for a randomized, placebo-controlled, double-blind multiple ascending dose with RO4917523 to evaluate safety and tolerability, PK and exploratory efficacy and pharmacodynamic effects. Seaside Therapeutics completed single and multiple oral dose clinical trials with STX107 in normal volunteers to assess safety and tolerability, PK and exploratory pharmacodynamic effects. The Hoffman La-Roche trial in FXS is not yet completed, and the Seaside Therapeutics phase I trials have not been reported.

### 15.4.3 GABA<sub>B</sub> Agonists

The importance of  $\gamma$ -aminobutyric acid (GABA) inhibitory transmission in FXS and ASD has recently emerged. The balance of neurotransmission in FXS and ASD is skewed towards an abnormally high ratio of excitatory to inhibitory (E:I) signalling, which may account for many of the clinical and neurobiological characteristics of ASD.<sup>133</sup> In FXS, data from the *Fmr1*-deficient mouse demonstrates extensive deficiencies in GABA-mediated inhibitory neurotransmission, and in the cellular machinery necessary to support it.<sup>134</sup> This deficiency is particularly pronounced in the amygdala, which is central to affective cognition. The abnormalities in mouse amygdala are paralleled by observations in human patients with FXS, who show excessive activation of the amygdala during social cognitive tasks.<sup>135</sup> GABA receptor agonists may therefore present a therapeutic solution to modulating the abnormal E:I signalling in ASD. Two distinct types of GABA receptors mediate inhibitory tone in the brain. Ionotropic GABA<sub>A</sub> receptors directly inhibit neurotransmission through their hyperpolarizing Cl<sup>−</sup> conductance, while metabotropic GABA<sub>B</sub> receptors alter cell signalling through release of G $\beta\gamma$  and G $\alpha_i$ /G $\alpha_o$  subunits. Unlike GABA<sub>A</sub> receptor agonists (*e.g.* benzodiazepines), which are prone to amnesic and sedative effects, GABA<sub>B</sub> receptor agonists may provide a more relevant target by reducing excitation while limiting mGluR5 signalling. Agonism of pre-synaptic GABA<sub>B</sub> receptors reduces glutamate release,<sup>136</sup> and consequently may reduce mGluR5 activation. Furthermore, agonism of post-synaptic GABA<sub>B</sub> receptors reduces cell excitability by activating K<sup>+</sup> channels<sup>137</sup> and may limit synaptic Ca<sup>2+</sup> signals by reducing Protein Kinase A activity.<sup>138</sup> Through these mechanisms, GABA<sub>B</sub> receptor agonism may target synaptic excitability and the mGluR5 pathway, and ultimately ameliorate the clinical impairments associated with FXS and ASD.

#### 15.4.4 STX209 in FXS and ASD

One GABA<sub>B</sub> receptor agonist is currently in clinical development for ASD, and for FXS as well. STX209, also known as arbaclofen, is the active enantiomer of racemic baclofen, which was approved about 30 years ago for the treatment of

spasticity.<sup>139</sup> The motivations for studying STX209 in ASD include the scientific rationale described above and data from animal models of FXS. In the FXS mouse model, for example, treatment with STX209 is associated with improvements in an array of abnormal phenotypes, including neurobehavioural endpoints such as audiogenic seizures and marble-burying, as well as disease-modifying endpoints such as modulation of protein synthesis, and rescue of dendritic spine morphology.<sup>140,141</sup>

In a double-blind, placebo-controlled, crossover study of STX209, sponsored by Seaside Therapeutics in 63 subjects with FXS, 4 weeks of drug treatment was associated with statistically significant improvement on parent-rated problem behaviours, and with trends for improvement on multiple global clinical ratings.<sup>142</sup> In a *post hoc* analysis on the subset of 27 subjects who had baseline social impairments that were higher than average for the FXS population as a whole, improvement was also seen in social function, a core deficit of both FXS and ASD. These subjects showed statistically significant improvement on two independent measures of social function (the ABC-Social Withdrawal scale and the Vineland Adaptive Behaviour Scale – Social Domain score) and on multiple global clinical ratings. Phase III studies are underway to confirm these findings.

Seaside Therapeutics also has completed an 8-week open-label study of STX209 in 32 subjects with ASD, aged 6–17 years.<sup>143</sup> These subjects showed statistically significant improvements on a broad battery of endpoints, including the ABC-Social Withdrawal scale, the ABC-Irritability scale, other scales of social function and multiple global clinical ratings. These results are being followed up by a large, controlled Phase IIb study in ASD.

## 15.4.5 Other Emerging Treatments

### 15.4.5.1 Oxytocin

Intra-nasal oxytocin, routinely used in women's reproductive medicine, has recently been tested in patients with high-functioning ASD or Asperger syndrome. In a double-blind, placebo-controlled, crossover study, 16 patients were tested and showed improved emotion recognition following oxytocin treatment compared with placebo.<sup>144</sup> In a separate double-blind, placebo-controlled study, the behavioural effects of oxytocin were tested on 13 male subjects with high-functioning ASD. In a simulated ball game patients exhibited stronger interactions with the most socially cooperative partner, reported enhanced feelings of trust and spent increased gazing time on socially informative regions of the face.<sup>145</sup> The evidence to date suggests oxytocin may be a targeted treatment for the social impairments associated with ASD and therefore could be targeted towards the higher functioning and Asperger syndrome patients. It is interesting to note that reduced GABAergic synapses were observed in cultured hippocampal neurons from oxytocin receptor-deficient mice suggesting an imbalance in GABA/glutamate transmission may also be a contributing factor to the deficits in social recognition, increased aggression and seizure susceptibility.<sup>146</sup>

### 15.4.5.2 *Rapamycin in TSC*

Because the loss-of-function mutations in TSC1/2 result in constitutive activation of mTOR, which is thought to underlie disease etiology, inhibiting mTOR is a reasonable approach to correcting aspects of this disorder. Rapamycin (sirolimus, everolimus), is an mTOR inhibitor approved for use as an immunosuppressive agent in organ transplant and as an anti-proliferative agent on coated vascular stents. Oral rapamycin has also been used to treat TSC tumours and showed some benefit in these early clinical studies.<sup>147–149</sup> Additional work continues to test rapamycin efficacy in reducing TSC tumours. Current studies are also examining the effects of rapamycin on cognition in TSC patients. Novartis Pharmaceuticals is conducting a phase II trial to test RAD001 (everolimus) in a randomized, double-blind study testing effects on cognition in TSC patients between the ages of 6 and 21 years, but the potential for improvements in autistic behaviour and seizures will be examined using secondary outcome measures (clinicaltrials.gov).

## 15.5 Clinical Outcome Measures

Perhaps the largest challenge facing the clinical development of therapeutics for ASD is the lack of outcome measures that are proven to be both valid and sensitive to change. While the DSM-IV, ADOS and ADI-R provide a widely accepted gold standard for diagnosis,<sup>150</sup> there is no consensus on the best instruments for quantitating the severity of the core symptoms of ASD, and the sensitivity to change of these severity rating instruments is largely unknown.<sup>151</sup> The outcome measures that have been used to demonstrate the efficacy of behavioural therapeutic approaches for ASD, which is more widely accepted than for any other treatment approach, have included general measures of communication, intelligence, educational achievement and diagnostic classification, rather than quantitative measures of autism severity.

A recent demonstration of the efficacy of a broad educational program in ASD is illustrative of current strategies for assessing outcomes.<sup>152</sup> This study compared the 1- and 2-year outcomes of children who received a specially designed educational program *versus* those who received standard community intervention. Children receiving the new educational program showed improvements in IQ (Mullen Scales of Early Learning), in functional, adaptive behaviour (Vineland Adaptive Behaviours Scales), and changes in their DSM-IV diagnostic classification (from “Autistic disorder” to the less severe “pervasive developmental disorder – not otherwise specified”). Improvement was not seen on a measure of repetitive and restricted behaviours. Improvements in IQ and in functional adaptive behaviour are certainly felt to be of clinical value, but these constructs are not specific to ASD, though functional adaptive behaviour has been shown to correlate with the severity of social symptoms in ASD.

The wide range of symptoms in ASD contributes to the challenge of constructing a valid and useful outcome measure. For example, the impairment



of communicative skills in ASD can range from the complete lack of either verbal or non-verbal communication in the lowest-functioning individuals with ASD, to subtle abnormalities of prosody or conversational skill, or difficulties in understanding body language, in higher-functioning individuals. The assessment of social skills is similarly challenged, ranging from the absence of interpersonal engagement, to difficulties in establishing and maintaining close friendships. This problem is compounded by the fact that ASD is still primarily diagnosed in the paediatric age range, where social and communication skills normally show large variability as a function of typical development.

Attempts have been made to adapt the ADOS from its current diagnostic use to become a measure of change in symptom severity, but the successful use of this metric in clinical trials has not yet been reported.<sup>153</sup> The ADI-R, which is designed to establish whether an individual has ever met criteria for autism during their lifetime, is by nature not readily adaptable for use as a measure of change. There do exist a few measures (*e.g.* the Pervasive Developmental Disorder – Behavioural Index) designed to quantitate symptom severity in ASD, and to assess change in severity,<sup>154</sup> but their validity and sensitivity to change have not yet been fully tested.

Drug studies to date have focused most commonly on the associated, non-core symptoms of ASD, especially irritability.<sup>155</sup> The ABC-Irritability scale (officially, the “Irritability/Agitation/Crying” subscale) is clearly sensitive to change, having shown effects with risperidone, aripiprazole and other drugs in ASD. The other subscales of the ABC include “Lethargy/Social Withdrawal”, “Stereotypic Behaviour”, “Hyperactivity/Noncompliance” and “Inappropriate Speech”. Hyperactivity is not a core symptom of ASD, and the utility in ASD of this ABC subscale relative to other instruments assessing hyperactivity and compliance has never been studied.

The other three subscales of the ABC are relevant to the core symptoms of ASD, but they do not reflect the full scope of core symptoms. For example, the Inappropriate Speech subscale touches on deviant behaviours such as echolalia, but does not begin to assess symptoms such as the absence of functional, communicative language. The ABC also includes items on certain behaviours, such as self-injury, that are not diagnostically specific to ASD. These caveats should not be surprising, since the ABC was not designed specifically for the assessment of ASD, and has never been validated in ASD. The ABC manual further recommends explicitly against the calculation of a summary “ABC-Total” score that combines the individual subscale scores, and that might be hoped to provide a broader characterization of ASD symptoms than any single subscale. This is because there are many items across the subscales of opposite valence (*e.g.* “Does nothing but sit and watch” from the Social Withdrawal subscale *vs.* “Cannot sit still” from the Hyperactivity subscale), such that a single behavioural change could increase one subscale score while decreasing another.

Several drug studies also have focused on repetitive behaviours, using an array of outcome measures, including the ABC-Stereotypic Behaviour subscale, the Repetitive Behaviour Rating Scale, and the Children’s Yale–Brown Obsessive-Compulsive Scale Modified for Pervasive Developmental



Disorders.<sup>156</sup> Beneficial drug effects have been found in some preliminary studies on these scales, but subsequent definitive studies have not replicated these results (*e.g.* for citalopram). At this time, these failures could be attributed to true lack of drug effect or psychometric deficiencies of the outcome measures, or both.

## 15.6 Biomarkers

Our lack of understanding of the core pathophysiologic mechanisms leading to altered brain function in CNS disorders, in general, and in autism in particular, necessitates a diverse approach to biomarker discovery. That, coupled to significant advances in molecular techniques and brain imaging methods, has further widened the lens of biomarker discovery. The most significant challenge to identifying biomarkers of ASD is the heterogeneity of the patient population. Individuals with autism present with a subset of symptoms: even within a single defined genetic abnormality, symptom variations exist. Markers to facilitate an autism diagnosis, aid patient selection into clinical trials and measure drug efficacy and safety are predicted to reduce drug development time and increase patient responsiveness to therapies.

The primary role for pharmacodynamic biomarkers of ASD would provide a short-term measure of drug efficacy or safety that may be used as a surrogate endpoint for the outcome measures of behavioural assessment. In addition to surrogate biomarkers that measure the patient response to treatment, predictive biomarkers are necessary to maximize safety and efficacy by identifying those patients more likely to show a favourable response to treatment. The ultimate goal is an objective measure of autism.

Molecular measures of disease state or drug treatment response have broad applications in medicine, in particular cardiovascular and cancer medicines. Advances in DNA sequencing methods combined with increasingly sophisticated molecular pathway analyses has centred investigations on genes and gene expression (including mRNA and micro-RNA), and protein, and largely focuses on accessible, biological fluids; blood, urine and cerebral spinal fluid. Although many questions need to be addressed before these types of measures can be applied to neurologic conditions such as autism, the most pressing question is whether peripheral blood biomarkers of CNS disorders exist. Profiling peripheral blood mRNA and proteomic analyses of patient plasma suggest that peripheral markers of CNS disorders can be found.<sup>157</sup> Whether these biomarkers or bio-signatures are specific to ASD and will show treatment effects remains to be determined.

Neurobehavioral biomarkers include eye gaze patterns when viewing socially relevant visual stimuli, the ability to rapidly switch attention across multiple streams of simultaneously presented stimuli, magneto- and electrophysiologic responses to auditory/verbal or face/visual stimuli, and cortical connectivity patterns observed on magnetic resonance imaging (MRI). Other clinical or neuroanatomic abnormalities in ASD have been found independent of engagement in any behavioural task. Examples of these include neuroanatomic

differences on MRI, and simple head circumference differences in comparison to typically developing children.

Research to date has not established whether any of these potential neuro-behavioral biomarkers have utility in drug development. In particular, there has been essentially no research investigating whether these measures are dynamic, showing change in association with drug treatment, or in association with clinical improvement. Research has only recently begun on the question of typical age-related changes in these phenomena in ASD.

A reservation regarding the neurobehavioral biomarkers is that none has been clearly related to the cellular and molecular pathophysiology of ASD. One exception might be found in the response of individuals with ASD to transcranial magnetic stimulation (TMS). TMS is used to measure changes in cortical synaptic plasticity in patients and, although the research on TMS in ASD is only in its earliest stages, it has been suggested that cortical plasticity is abnormal in ASD. The hypothesis that many cases of ASD are attributable to synaptic pathophysiology, discussed above, combined with research in healthy controls showing that TMS can be modulated by drug treatment, leads to the possibility that TMS might have utility as a neurobehavioral biomarker in drug development for ASD.

More generally, clinical measures of synaptic plasticity should be considered for deeper examination in ASD. The results of several studies have shown that perceptual adaptation to tactile stimulation, presumably also an expression of synaptic plasticity, is abnormal in ASD. This measure also can be modulated by drug treatment, again raising the possibility that it could be useful as a neurobehavioral biomarker for ASD drug development.

## 15.7 Conclusions

The past decade has seen enormous strides in autism research. The increased public awareness of ASD and more accurate diagnoses provided a greater understanding of disease prevalence. This, in turn, has increased attention towards improving educational and behavioural therapies, and developing new treatments for autism. Further, an increased understanding of the neuro-pathophysiology causing autism provides a path forward to identify and develop novel therapies to treat the core symptoms of autism. These advances represent a paradigm shift in our thinking. Instead of managing the associated symptoms of ASD, the goal of translational research is to alter the course of disease using mechanism-based approaches.

## References

1. U. Frith, *Autism and Asperger Syndrome*, Cambridge University Press, Cambridge; New York, 1991.
2. L. Kanner, *Acta Paedopsychiatr.*, 1968, **35**, 100–136.

3. American Psychiatric Association and American Psychiatric Association, Task Force on DSM-IV, *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR*, 4th edn, American Psychiatric Association, Washington, DC, 2000.
4. World Health Organization, *The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research*, World Health Organization, Geneva, 1993.
5. A. P. Association, *DSM-5 Development*, [www.dsm5.org](http://www.dsm5.org), accessed July 20, 2011, 2011.
6. C. Lord, *Psychiatr. Clin. North Am.*, 1991, **14**, 69–80.
7. E. Hollander, A. Kolevzon and J. T. Coyle, *Textbook of Autism Spectrum Disorders*, American Psychiatric Publishing, Inc., Washington, DC, 1st edn, 2011.
8. M. D. Kogan, S. J. Blumberg, L. A. Schieve, C. A. Boyle, J. M. Perrin, R. M. Ghandour, G. K. Singh, B. B. Strickland, E. Trevathan and P. C. van Dyck, *Pediatrics*, 2009, **124**, 1395–1403.
9. *MMWR Surveill., Summ.*, 2012, **61**, 1–19.
10. M. Yeargin-Allsopp, C. Rice, T. Karapurkar, N. Doernberg, C. Boyle and C. Murphy, *JAMA*, 2003, **289**, 49–55.
11. M. King and P. Bearman, *Int. J. Epidemiol.*, 2009, **38**, 1224–1234.
12. T. S. Brugha, S. McManus, J. Bankart, F. Scott, S. Purdon, J. Smith, P. Bebbington, R. Jenkins and H. Meltzer, *Arch. Gen. Psychiatry*, 2011, **68**, 459–465.
13. Y. S. Kim, B. L. Leventhal, Y. J. Koh, E. Fombonne, E. Laska, E. C. Lim, K. A. Cheon, S. J. Kim, Y. K. Kim, H. Lee, D. H. Song and R. R. Grinker, *Am. J. Psychiatry*, 2011, **168**(9), 904.
14. P. J. Landrigan, *Curr. Opin. Pediatr.*, 2010, **22**, 219–225.
15. The Autism Genome Project Consortium, *Nature*, 2010, **466**, 368–372.
16. C. Betancur, *Brain Res.*, 2011, **1380**, 42–77.
17. E. R. Ritvo, L. B. Jorde, A. Mason-Brothers, B. J. Freeman, C. Pingree, M. B. Jones, W. M. McMahon, P. B. Petersen, W. R. Jenson and A. Mo, *Am. J. Psychiatry*, 1989, **146**, 1032–1036.
18. S. L. Smalley, R. F. Asarnow and M. A. Spence, *Arch. Gen. Psychiatry*, 1988, **45**, 953–961.
19. A. de la Chapelle, R. Herva, M. Koivisto and P. Aula, *Hum. Genet.*, 1981, **57**, 253–256.
20. T. Lukusa, J. R. Vermeesch, M. Holvoet, J. P. Fryns and K. Devriendt, *Genet. Couns.*, 2004, **15**, 293–301.
21. K. B. Nielsen, N. Tommerup, H. V. Dyggve and C. Schou, *Hum. Genet.*, 1982, **61**, 113–117.
22. D. J. Wolff, K. Clifton, C. Karr and J. Charles, *Genet. Med.*, 2002, **4**, 10–14.
23. E. H. Cook, V. Lindgren, B. L. Leventhal, R. Courchesne, A. Lincoln, C. Shulman, C. Lord and E. Courchesne, *Am. J. Hum. Genet.*, 1997, **60**, 928–934.
24. A. Hogart, D. Wu, J. M. LaSalle and N. C. Schanen, *Neurobiol. Dis.*, 2010, **38**, 181–191.

25. A. Ingason, G. Kirov, I. Giegling, T. Hansen, A. R. Isles, K. D. Jakobsen, K. T. Kristinsson, L. le Roux, O. Gustafsson, N. Craddock, H. Möller, A. McQuillin, P. Muglia, S. Cichon, M. Rietschel, R. A. Ophoff, S. Djurovic, O. A. Andreassen, O. P. Pietiläinen, L. Peltonen, E. Dempster, D. A. Collier, D. St Clair, H. B. Rasmussen, B. Y. Glenthøj, L. A. Kiemeny, B. Franke, S. Tosato, C. Bonetto, E. Saemundsen, S. J. Hreidarsson, GROUP Investigators, M. M. Nöthen, H. Gurling, M. C. O'Donovan, M. J. Owen, E. Sigurdsson, H. Petursson, H. Stefansson, D. Rujescu, K. Stefansson and T. Werge, *Am. J. Psychiatry*, 2011, **168**, 408–417.
26. C. Depienne, D. Moreno-De-Luca, D. Heron, D. Bouteiller, A. Gennetier, R. Delorme, P. Chaste, J. P. Siffroi, S. Chantot-Bastaraud, B. Benyahia, O. Trouillard, G. Nygren, S. Kopp, M. Johansson, M. Rastam, L. Burglen, E. Leguern, A. Verloes, M. Leboyer, A. Brice, C. Gillberg and C. Betancur, *Biol. Psychiatry*, 2009, **66**(4), 349.
27. B. Horsthemke and K. Buiting, *Adv. Genet.*, 2008, **61**, 225–246.
28. A. Lewis and W. Reik, *Cytogenet. Genome Res.*, 2006, **113**, 81–89.
29. T. Kishino, M. Lalande and J. Wagstaff, *Nat. Genet.*, 1997, **15**, 70–73.
30. C. Rougeulle, H. Glatt and M. Lalande, *Nat. Genet.*, 1997, **17**, 14–15.
31. C. Rougeulle, C. Cardoso, M. Fontes, L. Colleaux and M. Lalande, *Nat. Genet.*, 1998, **19**, 15–16.
32. C. M. Durand, C. Betancur, T. M. Boeckers, J. Bockmann, P. Chaste, F. Fauchereau, G. Nygren, M. Rastam, I. C. Gillberg, H. Anckarsäter, E. Sponheim, H. Goubran-Botros, R. Delorme, N. Chabane, M. C. Mouren-Simeoni, P. de Mas, E. Bieth, B. Rogé, D. Héron, L. Burglen, C. Gillberg, M. Leboyer and T. Bourgeron, *Nat. Genet.*, 2007, **39**, 25–27.
33. G. Roussignol, F. Ango, S. Romorini, J. C. Tu, C. Sala, P. F. Worley, J. Bockaert and L. Fagni, *J. Neurosci.*, 2005, **25**, 3560–3570.
34. X. Wang, P. A. McCoy, R. M. Rodriguiz, Y. Pan, H. S. Je, A. C. Roberts, C. Kim, J. Berrios, J. S. Colvin, D. Bousquet-Moore, I. Lorenzo, G. Wu, R. J. Weinberg, M. D. Ehlers, B. D. Philpot, A. Beaudet, W. C. Wetsel and Y. H. Jiang, *Hum. Mol. Genet.*, 2011, **20**, 3093–3108.
35. D. F. Conrad, D. Pinto, R. Redon, L. Feuk, O. Gokcumen, Y. Zhang, J. Aerts, T. D. Andrews, C. Barnes, P. Campbell, T. Fitzgerald, M. Hu, C. H. Ihm, K. Kristiansson, D. G. Macarthur, J. R. Macdonald, I. Onyiah, A. W. Pang, S. Robson, K. Stirrups, A. Valsesia, K. Walter, J. Wei, C. Tyler-Smith, N. P. Carter, C. Lee, S. W. Scherer and M. E. Hurles, *Nature*, 2010, **464**, 704–712.
36. J. Sebat, B. Lakshmi, D. Malhotra, J. Troge, C. Lese-Martin, T. Walsh, B. Yamrom, S. Yoon, A. Krasnitz, J. Kendall, A. Leotta, D. Pai, R. Zhang, Y. H. Lee, J. Hicks, S. J. Spence, A. T. Lee, K. Puura, T. Lehtimäki, D. Ledbetter, P. K. Gregersen, J. Bregman, J. S. Sutcliffe, V. Jobanputra, W. Chung, D. Warburton, M. C. King, D. Skuse, D. H. Geschwind, T. C. Gilliam, K. Ye and M. Wigler, *Science*, 2007, **316**, 445–449.
37. L. A. Weiss, D. E. Arking, M. J. Daly and A. Chakravarti, *Nature*, 2009, **461**, 802–808.

38. C. R. Marshall, A. Noor, J. B. Vincent, A. C. Lionel, L. Feuk, J. Skaug, M. Shago, R. Moessner, D. Pinto, Y. Ren, B. Thiruvahindrapduram, A. Fiebig, S. Schreiber, J. Friedman, C. E. Ketelaars, Y. J. Vos, C. Ficicioglu, S. Kirkpatrick, R. Nicolson, L. Sloman, A. Summers, C. A. Gibbons, A. Teebi, D. Chitayat, R. Weksberg, C. Vardy, V. Crosbie, S. Luscombe, R. Baatjes, L. Zwaigenbaum, W. Roberts, B. Fernandez, P. Szatmari and S. W. Scherer, *Am. J. Hum. Genet.*, 2008, **82**, 477–488.
39. R. A. Kumar, S. KaraMohamed, J. Sudi, D. F. Conrad, C. Brune, J. A. Badner, T. C. Gilliam, N. J. Nowak, E. H. Cook, Jr, W. B. Dobyns and S. L. Christian, *Hum. Mol. Genet.*, 2008, **17**, 628–638.
40. A. Bremer, M. Giacobini, M. Eriksson, P. Gustavsson, V. Nordin, E. Fernell, C. Gillberg, A. Nordgren, A. Uppstromer, B. M. Anderlid, M. Nordenskjold and J. Schoumans, *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 2011, **156**, 115–124.
41. B. A. Fernandez, W. Roberts, B. Chung, R. Weksberg, S. Meyn, P. Szatmari, A. M. Joseph-George, S. Mackay, K. Whitten, B. Noble, C. Vardy, V. Crosbie, S. Luscombe, E. Tucker, L. Turner, C. R. Marshall and S. W. Scherer, *J. Med. Genet.*, 2010, **47**, 195–203.
42. S. J. Sanders, A. G. Ercan-Sencicek, V. Hus, R. Luo, M. T. Murtha, D. Moreno-De-Luca, S. H. Chu, M. P. Moreau, A. R. Gupta, S. A. Thomson, C. E. Mason, K. Bilguvar, P. B. Celestino-Soper, M. Choi, E. L. Crawford, L. Davis, N. R. Davis Wright, R. M. Dhodapkar, M. Dicola, N. M. Dilullo, T. V. Fernandez, V. Fielding-Singh, D. O. Fishman, S. Frahm, R. Garagaloyan, G. S. Goh, S. Kammela, L. Klei, J. K. Lowe, S. C. Lund, A. D. McGrew, K. A. Meyer, W. J. Moffat, J. D. Murdoch, B. J. O’Roak, G. T. Ober, R. S. Pottenger, M. J. Raubeson, Y. Song, Q. Wang, B. L. Yaspan, T. W. Yu, I. R. Yurkiewicz, A. L. Beaudet, R. M. Cantor, M. Curland, D. E. Grice, M. Gunel, R. P. Lifton, S. M. Mane, D. M. Martin, C. A. Shaw, M. Sheldon, J. A. Tischfield, C. A. Walsh, E. M. Morrow, D. H. Ledbetter, E. Fombonne, C. Lord, C. L. Martin, A. I. Brooks, J. S. Sutcliffe, E. H. Cook, Jr, D. Geschwind, K. Roeder and B. Devlin, and M. W. State, *Neuron*, 2011, **70**, 863–885.
43. K. M. Walsh and M. B. Bracken, *Genet. Med.*, 2011, **13**, 377–384.
44. D. Levy, M. Ronemus, B. Yamrom, Y. H. Lee, A. Leotta, J. Kendall, S. Marks, B. Lakshmi, D. Pai, K. Ye, A. Buja, A. Krieger, S. Yoon, J. Troge, L. Rodgers, I. Iossifov and M. Wigler, *Neuron*, 2011, **70**, 886–897.
45. C. Modahl, L. Green, D. Fein, M. Morris, L. Waterhouse, C. Feinstein and H. Levin, *Biol. Psychiatry*, 1998, **43**, 270–277.
46. L. Green, D. Fein, C. Modahl, C. Feinstein, L. Waterhouse and M. Morris, *Biol. Psychiatry*, 2001, **50**, 609–613.
47. S. Wu, M. Jia, Y. Ruan, J. Liu, Y. Guo, M. Shuang, X. Gong, Y. Zhang, X. Yang and D. Zhang, *Biol. Psychiatry*, 2005, **58**, 74–77.
48. T. Ylisaukko-oja, M. Alarcon, R. M. Cantor, M. Auranen, R. Vanhala, E. Kempas, L. von Wendt, I. Jarvela, D. H. Geschwind and L. Peltonen, *Ann. Neurol.*, 2006, **59**, 145–155.

49. D. B. Campbell, D. Datta, S. T. Jones, E. Batey Lee, J. S. Sutcliffe, E. A. Hammock and P. Levitt, *J. Neurodev. Disord.*, 2011, **3**, 101–112.
50. Y. M. Takayanagi, I. F. Bielsky, H. E. Ross, M. Kawamata, T. Onaka, T. Yanagisawa, T. Kimura, M. M. Matzuk, L. J. Young and K. Nishimori, *Proc. Natl Acad. Sci. USA*, 2005, **44**, 16096–16101.
51. K. Wang, H. Zhang, D. Ma, M. Bucan, J. T. Glessner, B. S. Abrahams, D. Salyakina, M. Imielinski, J. P. Bradfield, P. M. Sleiman, C. E. Kim, C. Hou, E. Frackelton, R. Chiavacci, N. Takahashi, T. Sakurai, E. Rappaport, C. M. Lajonchere, J. Munson, A. Estes, O. Korvatska, J. Piven, L. I. Sonnenblick, A. I. Alvarez Retuerto, E. I. Herman, H. Dong, T. Hutman, M. Sigman, S. Ozonoff, A. Klin, T. Owley, J. A. Sweeney, C. W. Brune, R. M. Cantor, R. Bernier, J. R. Gilbert, M. L. Cuccaro, W. M. McMahon, J. Miller, M. W. State, T. H. Wassink, H. Coon, S. E. Levy, R. T. Schultz, J. I. Nurnberger, J. L. Haines, J. Sutcliffe, E. H. Cook, N. J. Minshew, J. D. Buxbaum, G. Dawson, S. F. Grant, D. H. Geschwind, M. A. Pericak-Vance, G. D. Schellenberg and H. Hakonarson, *Nature*, 2009, **459**, 528–533.
52. R. Anney, L. Klei, D. Pinto, R. Regan, J. Conroy, T. R. Magalhaes, C. Correia, B. S. Abrahams, N. Sykes, A. T. Pagnamenta, J. Almeida, E. Bacchelli, A. J. Bailey, G. Baird, A. Battaglia, T. Berney, N. Bolshakova, S. Bolte, P. F. Bolton, T. Bourgeron, S. Brennan, J. Brian, A. R. Carson, G. Casallo, J. Casey, S. H. Chu, L. Cochrane, C. Corsello, E. L. Crawford, A. Crossett, G. Dawson, M. de Jonge, R. Delorme, I. Drmic, E. Duketis, F. Duque, A. Estes, P. Farrar, B. A. Fernandez, S. E. Folstein, E. Fombonne, C. M. Freitag, J. Gilbert, C. Gillberg, J. T. Glessner, J. Goldberg, J. Green, S. J. Guter, H. Hakonarson, E. A. Heron, M. Hill, R. Holt, J. L. Howe, G. Hughes, V. Hus, R. Igliozzi, C. Kim, S. M. Klauck, A. Kolevzon, O. Korvatska, V. Kustanovich, C. M. Lajonchere, J. A. Lamb, M. Laskawiec, M. Leboyer, A. Le Couteur, B. L. Leventhal, A. C. Lionel, X. Q. Liu, C. Lord, L. Lotspeich, S. C. Lund, E. Maestrini, W. Mahoney, C. Mantoulan, C. R. Marshall, H. McConachie, C. J. McDougale, J. McGrath, W. M. McMahon, N. M. Melhem, A. Merikangas, O. Migita, N. J. Minshew, G. K. Mirza, J. Munson, S. F. Nelson, C. Noakes, A. Noor, G. Nygren, G. Oliveira, K. Papanikolaou, J. R. Parr, B. Parrini, T. Paton, A. Pickles, J. Piven, D. J. Posey, A. Poustka, F. Poustka, A. Prasad, J. Ragoussis, K. Renshaw, J. Rickaby, W. Roberts, K. Roeder, B. Roge, M. L. Rutter, L. J. Bierut, J. P. Rice, J. Salt, K. Sansom, D. Sato, R. Segurado, L. Senman, N. Shah, V. C. Sheffield, L. Soorya, I. Sousa, V. Stoppioni, C. Strawbridge, R. Tancredi, K. Tansey, B. Thiruvahindrapduram, A. P. Thompson, S. Thomson, A. Tryfon, J. Tsiantis, H. Van Engeland, J. B. Vincent, F. Volkmar, S. Wallace, K. Wang, Z. Wang, T. H. Wassink, K. Wing, K. Wittemeyer, S. Wood, B. L. Yaspan, D. Zurawiecki, L. Zwaigenbaum, C. Betancur, J. D. Buxbaum, R. M. Cantor, E. H. Cook, H. Coon, M. L. Cuccaro, L. Gallagher, D. H. Geschwind, M. Gill, J. L. Haines, J. Miller, A. P. Monaco, J. I. Nurnberger, Jr, A. D. Paterson, M. A. Pericak-Vance, G. D. Schellenberg, S. W. Scherer, J. S. Sutcliffe, P. Szatmari, A. M.



- Vicente, V. J. Vieland, E. M. Wijsman, B. Devlin, S. Ennis and J. Hallmayer, *Hum. Mol. Genet.*, 2010, **19**, 4072–4082.
53. A. Itsara, H. Wu, J. D. Smith, D. A. Nickerson, I. Romieu, S. J. London and E. E. Eichler, *Genome Res.*, 2010, **20**, 1469–1481.
  54. D. Pinto, A. T. Pagnamenta, L. Klei, R. Anney, D. Merico, R. Regan, J. Conroy, T. R. Magalhaes, C. Correia, B. S. Abrahams, J. Almeida, E. Bacchelli, G. D. Bader, A. J. Bailey, G. Baird, A. Battaglia, T. Berney, N. Bolshakova, S. Bolte, P. F. Bolton, T. Bourgeron, S. Brennan, J. Brian, S. E. Bryson, A. R. Carson, G. Casallo, J. Casey, B. H. Chung, L. Cochrane, C. Corsello, E. L. Crawford, A. Crossett, C. Cytrynbaum, G. Dawson, M. de Jonge, R. Delorme, I. Drmic, E. Duketis, F. Duque, A. Estes, P. Farrar, B. A. Fernandez, S. E. Folstein, E. Fombonne, C. M. Freitag, J. Gilbert, C. Gillberg, J. T. Glessner, J. Goldberg, A. Green, J. Green, S. J. Guter, H. Hakonarson, E. A. Heron, M. Hill, R. Holt, J. L. Howe, G. Hughes, V. Hus, R. Igliozzi, C. Kim, S. M. Klauck, A. Klevzon, O. Korvatska, V. Kustanovich, C. M. Lajonchere, J. A. Lamb, M. Laskawiec, M. Leboyer, A. Le Couteur, B. L. Leventhal, A. C. Lionel, X. Q. Liu, C. Lord, L. Lotspeich, S. C. Lund, E. Maestrini, W. Mahoney, C. Mantoulan, C. R. Marshall, H. McConachie, C. J. McDougale, J. McGrath, W. M. McMahon, A. Merikangas, O. Migita, N. J. Minshew, G. K. Mirza, J. Munson, S. F. Nelson, C. Noakes, A. Noor, G. Nygren, G. Oliveira, K. Papanikolaou, J. R. Parr, B. Parrini, T. Paton, A. Pickles, M. Pilorge, J. Piven, C. P. Ponting, D. J. Posey, A. Poustka, F. Poustka, A. Prasad, J. Ragoussis, K. Renshaw, J. Rickaby, W. Roberts, K. Roeder, B. Roge, M. L. Rutter, L. J. Bierut, J. P. Rice, J. Salt, K. Sansom, D. Sato, R. Segurado, A. F. Sequeira, L. Senman, N. Shah, V. C. Sheffield, L. Soorya, I. Sousa, O. Stein, N. Sykes, V. Stoppioni, C. Strawbridge, R. Tancredi, K. Tansey, B. Thiruvahindrapduram, A. P. Thompson, S. Thomson, A. Tryfon, J. Tsiantis, H. Van Engeland, J. B. Vincent, F. Volkmar, S. Wallace, K. Wang, Z. Wang, T. H. Wassink, C. Webber, R. Weksberg, K. Wing, K. Wittemeyer, S. Wood, J. Wu, B. L. Yaspan, D. Zurawiecki, L. Zwaigenbaum, J. D. Buxbaum, R. M. Cantor, E. H. Cook, H. Coon, M. L. Cuccaro, B. Devlin, S. Ennis, L. Gallagher, D. H. Geschwind, M. Gill, J. L. Haines, J. Hallmayer, J. Miller, A. P. Monaco, J. I. Nurnberger, Jr, A. D. Paterson, M. A. Pericak-Vance, G. D. Schellenberg, P. Szatmari, A. M. Vicente, V. J. Vieland, E. M. Wijsman, S. W. Scherer, J. S. Sutcliffe and C. Betancur, *Nature*, 2010, **466**, 368–372.
  55. S. R. Gilman, I. Iossifov, D. Levy, M. Ronemus, M. Wigler and D. Vitkup, *Neuron*, 2011, **70**, 898–907.
  56. C. Mazzucchelli, C. Vantaggiato, A. Ciamei, S. Fasano, P. Pakhotin, W. Krezel, H. Welzl, D. P. Wolfer, G. Pages, O. Valverde, A. Marowsky, A. Porrazzo, P. C. Orban, R. Maldonado, M. U. Ehrenguber, V. Cestari, H. P. Lipp, P. F. Chapman, J. Pouyssegur and R. Brambilla, *Neuron*, 2002, **34**, 807–820.
  57. Y. A. Ushkaryov, A. G. Petrenko, M. Geppert and T. C. Südhof, *Science*, 1992, **257**, 50–56.



58. F. Varoqueaux, G. Aramuni, R. L. Rawson, R. Mohrmann, M. Missler, K. Gottmann, W. Zhang, T. C. Südhof and N. Brose, *Neuron*, 2006, **51**, 741–754.
59. E. R. Graf, X. Zhang, S. X. Jin, M. W. Linhoff and A. M. Craig, *Cell*, 2004, **119**, 1013–1026.
60. N. S. Thomas, A. J. Sharp, C. E. Browne, D. Skuse, C. Hardie and N. R. Dennis, *Hum. Genet.*, 1999, **104**, 43–48.
61. Y. Shao, C. M. Wolpert, K. L. Raiford, M. M. Menold, S. L. Donnelly, S. A. Ravan, M. P. Bass, C. McClain, L. von Wendt, J. M. Vance, R. H. Abramson, H. H. Wright, A. Ashley-Koch, J. R. Gilbert, R. G. DeLong, M. L. Cuccaro and M. A. Pericak-Vance, *Am. J. Med. Genet.*, 2002, **114**, 99–105.
62. S. Jamain, H. Quach, C. Betancur, M. Råstam, C. Colineaux, I. C. Gillberg, H. Soderstrom, B. Giros, M. Leboyer, C. Gillberg and T. Bourgeron, *Nat. Genet.*, 2003, **34**, 27–29.
63. D. Comoletti, A. De Jaco, L. L. Jennings, R. E. Flynn, G. Gaietta, I. Tsigelny, M. H. Ellisman and P. Taylor, *J. Neurosci.*, 2004, **24**, 4889–4893.
64. T. Walsh, J. M. McClellan and S. E. McCarthy, *Science*, 2008, **320**, 539–543.
65. G. Kirov, D. Gumus and W. Chen, *Hum. Mol. Genet.*, 2008, **17**, 458–465.
66. J. Feng, R. Schroer and J. Yan, *Neurosci. Lett.*, 2006, **409**, 10–13.
67. A. G. P. Consortium, *Nat. Genet.*, 2007, **39**, 319–328.
68. H. Daoud, F. Bonnet-Brilhault, S. Védrine, M. V. Demattéi, P. Vourc'h, N. Bayou, C. R. Andres, C. Barthélémy, F. Laumonnie and S. Briault, *Biol. Psychiatry*, 2009, **66**(10), 906–910. Epub 2009 Jul 9, 2009, **66**, 906–910.
69. D. H. Geschwind, *Biol. Psychiatry*, 2009, **66**, 904–905.
70. M. K. Belmonte and T. Bourgeron, *Nat. Neurosci.*, 2006, **9**, 1221–1225.
71. D. I. Zafeiriou, A. Ververi and E. Vargiami, *Brain Dev.*, 2007, **29**, 257–272.
72. A. L. Reiss and S. S. Hall, *Child Adolesc. Psychiatr. Clin. N. Am.*, 2007, **16**, 663–675.
73. O. Penagarikano, J. G. Mulle and S. T. Warren, *Annu. Rev. Genomics Hum. Genet.*, 2007, **8**, 109–129.
74. I. Plante, L. Davidovic, D. L. Ouellet, L. A. Gobeil, S. Tremblay, E. W. Khandjian and P. Provost, *J. Biomed. Biotechnol.*, 2006, **2006**, 64347.
75. A. Ishizuka, M. C. Siomi and H. Siomi, *Genes Dev.*, 2002, **16**, 2497–2508.
76. A. A. Caudy, M. Myers, G. J. Hannon and S. M. Hammond, *Genes Dev.*, 2002, **16**, 2491–2496.
77. A. A. Caudy and G. J. Hannon, *Methods Mol. Biol.*, 2004, **265**, 59–72.
78. V. Brown, P. Jin, S. Ceman, J. C. Darnell, W. T. O'Donnell, S. A. Tenenbaum, X. Jin, Y. Feng, K. D. Wilkinson, J. D. Keene, R. B. Darnell and S. T. Warren, *Cell*, 2001, **107**, 477–487.
79. The Dutch Belgian Fragile X Consortium, *Cell*, 1994, **78**, 23–33.
80. W. T. Greenough, A. Y. Klintsova, S. A. Irwin, R. Galvez, K. E. Bates and I. J. Weiler, *Proc. Natl Acad. Sci. USA*, 2001, **98**, 7101–7106.

81. S. W. Yun, J. Platholi, M. S. Flaherty, W. Fu, A. H. Kottmann and M. Toth, *Brain Res.*, 2006, **1110**, 159–165.
82. D. J. Kwiatkowski and B. D. Manning, *Hum. Mol. Genet.*, 2005, **14**, R251–R258.
83. M. Wiznitzer, *J. Child Neurol.*, 2004, **19**, 675–679.
84. D. Ehninger and A. J. Silva, *Trends Mol. Med.*, 2011, **17**, 78–87.
85. J. D. Richter and N. Sonenberg, *Nature*, 2005, **433**, 477–480.
86. S. F. Tavazoie, V. A. Alvarez, D. A. Ridenour, D. J. Kwiatkowski and B. L. Sabatini, *Nat. Neurosci.*, 2005, **8**, 1727–1734.
87. D. Ehninger, S. Han, C. Shilyansky, Y. Zhou, W. Li, D. J. Kwiatkowski, V. Ramesh and A. J. Silva, *Nat. Med.*, 2008, **14**, 843–848.
88. L. Meikle, D. M. Talos, H. Onda, K. Pollizzi, A. Rotenberg, M. Sahin, F. E. Jensen and D. J. Kwiatkowski, *J. Neurosci.*, 2007, **27**, 5546–5558.
89. H. S. Bateup, K. T. Takasaki, J. L. Saulnier, C. L. Denefrio and B. L. Sabatini, *J. Neurosci.*, 2011, **31**, 8862–8869.
90. E. A. Varga, M. Pastore, T. Prior, G. E. Herman and K. McBride, *Genet. Med.*, 2009, **11**(2), 111–117.
91. M. G. Butler, M. J. Dasouki, X. P. Zhou, Z. Talebizadeh, M. Brown, T. N. Takahashi, J. H. Miles, C. H. Wang, R. Stratton, R. Pilarski and C. Eng, *J. Med. Genet.*, 2005, **42**, 318–321.
92. L. C. Cantley, *Science*, 2002, **296**, 1655–1657.
93. J. D. Buxbaum, G. Cai, P. Chaste, G. Nygren, J. Goldsmith, J. Reichert, H. Anckarsater, M. Rastam, C. J. Smith, J. M. Silverman, E. Hollander, M. Leboyer, C. Gillberg, A. Verloes and C. Betancur, *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 2007, **144B**, 484–491.
94. J. Zhou, J. Blundell, S. Ogawa, C. H. Kwon, W. Zhang, C. Sinton, C. M. Powell and L. F. Parada, *J. Neurosci.*, 2009, **29**, 1773–1783.
95. C. H. Kwon, B. W. Luikart, C. M. Powell, J. Zhou, S. A. Matheny, W. Zhang, Y. Li, S. J. Baker and L. F. Parada, *Neuron*, 2006, **50**, 377–388.
96. B. Dasgupta, L. L. Dugan and D. H. Gutmann, *J. Neurosci.*, 2003, **23**, 8949–8954.
97. B. Dasgupta, Y. Yi, D. Y. Chen, J. D. Weber and D. H. Gutmann, *Cancer Res.*, 2005, **65**, 2755–2760.
98. S. V. Dindot, B. A. Antalffy, M. B. Bhattacharjee and A. L. Beaudet, *Hum. Mol. Genet.*, 2008, **17**, 111–118.
99. K. Yashiro, T. T. Riday, K. H. Condon, A. C. Roberts, D. R. Bernardo, R. Prakash, R. J. Weinberg, M. D. Ehlers and B. D. Philpot, *Nat. Neurosci.*, 2009, **12**, 777–783.
100. P. L. Greer, R. Hanayama, B. L. Bloodgood, A. R. Mardinly, D. M. Lipton, S. W. Flavell, T. K. Kim, E. C. Griffith, Z. Waldon, R. Maehr, H. L. Ploegh, S. Chowdhury, P. F. Worley, J. Steen and M. E. Greenberg, *Cell*, 2010, **140**, 704–716.
101. R. Klein, *Nat. Neurosci.*, 2009, **12**, 15–20.
102. S. S. Margolis, J. Salogiannis, D. M. Lipton, C. Mandel-Brehm, Z. P. Wills, A. R. Mardinly, L. Hu, P. L. Greer, J. B. Bikoff, H. Y. Ho, M. J. Soskis, M. Sahin and M. E. Greenberg, *Cell*, 2010, **143**, 442–455.

103. J. D. Shepherd and M. F. Bear, *Nat. Neurosci.*, 2011, **14**, 279–284.
104. M. Chahrouh, S. Y. Jung, C. Shaw, X. Zhou, S. T. Wong, J. Qin and H. Y. Zoghbi, *Science*, 2008, **320**, 1224–1229.
105. M. Chahrouh and H. Y. Zoghbi, *Neuron*, 2007, **56**, 422–437.
106. R. J. Kelleher, 3rd and M. F. Bear, *Cell*, 2008, **135**, 401–406.
107. S. Ricciardi, E. M. Boggio, S. Grosso, G. Lonetti, G. Forlani, G. Stefanelli, E. Calcagno, N. Morello, N. Landsberger, S. Biffo, T. Pizzorusso, M. Giustetto and V. Broccoli, *Hum. Mol. Genet.*, 2011, **20**, 1182–1196.
108. D. Tropea, E. Giacometti, N. R. Wilson, C. Beard, C. McCurry, D. D. Fu, R. Flannery, R. Jaenisch and M. Sur, *Proc. Natl Acad. Sci. USA*, 2009, **106**, 2029–2034.
109. G. M. van Woerden, K. D. Harris, M. R. Hojjati, R. M. Gustin, S. Qiu, R. de Avila Freire, Y. H. Jiang, Y. Elgersma and E. J. Weeber, *Nat. Neurosci.*, 2007, **10**, 280–282.
110. M. F. Bear, G. Dölen, E. Osterweil and N. Nagarajan, *Neuropsychopharmacology*, 2008, **33**, 84–87.
111. I. Voineagu, X. Wang, P. Johnston, J. K. Lowe, Y. Tian, S. Horvath, J. Mill, R. M. Cantor, B. J. Blencowe and D. H. Geschwind, *Nature*, 2011, **474**, 380–384.
112. K. Strömmland, V. Nordin, M. Miller, B. Akerström and C. Gillberg, *Dev. Med. Child Neurol.*, 1994, **36**, 351–356.
113. G. Williams, J. King, M. Cunningham, M. Stephan, B. Kerr and J. H. Hersh, *Dev. Med. Child Neurol.*, 2001, **43**, 202–206.
114. E. Kolozsi, R. N. Mackenzie and F. I. Roulet, D. deCatanzaro and J. A. Foster, *Neuroscience*, 2009, **163**, 1201–1210.
115. M. Eliassen, J. S. Tolstrup, A. M. Nybo Andersen, M. Grønbaek, J. Olsen and K. Strandberg-Larsen, *Int. J. Epidemiol.*, 2010, **39**, 1074–1081.
116. L. A. Croen, J. K. Grether, C. K. Yoshida, R. Odouli and V. Hendrick, *Arch. Gen. Psychiatry*, 2011, **38**(11), 1104.
117. M. D. Alter, R. Kharkar, K. E. Ramsey, D. W. Craig, R. D. Melmed, T. A. Grebe, R. C. Bay, S. Ober-Reynolds, J. Kirwan, J. J. Jones, J. B. Turner, R. Hen and D. A. Stephan, *PLoS One*, 2011, **6**, e16715.
118. J. Hallmayer, S. Cleveland, A. Torres, J. Phillips, B. Cohen, T. Torigoe, J. Miller, A. Fedeale, J. Collins, K. Smith, L. Lotspeich, L. A. Croen, S. Ozonoff, C. Lajonchere, J. K. Grether and N. Risch, *Arch. Gen. Psychiatry*, 2011, **68**(11), 1095.
119. D. S. Mandell, K. H. Morales, S. C. Marcus, A. C. Stahmer, J. Doshi and D. E. Polsky, *Pediatrics*, 2008, **121**, e441–e448.
120. L. C. Huffman, T. L. Sutcliffe, I. S. Tanner and H. M. Feldman, *J. Dev. Behav. Pediatr.*, 2011, **32**, 56–68.
121. K. Williams, D. M. Wheeler, N. Silove and P. Hazell, *Cochrane Database Syst. Rev.*, 2010, CD004677.
122. Food and Drug Administration, *FDA Online Label Repository*, labels.fda.gov, accessed July 20, 2011, 2011.
123. J. T. McCracken, J. McGough, B. Shah, P. Cronin, D. Hong, M. G. Aman, L. E. Arnold, R. Lindsay, P. Nash, J. Hollway, C. J. McDougle,

- D. Posey, N. Swiezy, A. Kohn, L. Scahill, A. Martin, K. Koenig, F. Volkmar, D. Carroll, A. Lancor, E. Tierney, J. Ghuman, N. M. Gonzalez, M. Grados, B. Vitiello, L. Ritz, M. Davies, J. Robinson and D. McMahon, *N. Engl. J. Med.*, 2002, **347**, 314–321.
124. R. Owen, L. Sikich, R. N. Marcus, P. Corey-Lisle, G. Manos, R. D. McQuade, W. H. Carson and R. L. Findling, *Pediatrics*, 2009, **124**, 1533–1540.
  125. C. A. Erickson, K. A. Stigler, D. J. Posey and C. J. McDougale, *Neurotherapeutics*, 2010, **7**, 258–263.
  126. M. F. Bear, in *Mechanistic Relationships between Development and Learning*, ed. T. J. Carew, R. Menzel and C. J. Schatz, John Wiley and Sons, New York, 1998, pp. 205–225.
  127. I. J. Weiler and W. T. Greenough, *Proc. Natl Acad. Sci. USA*, 1993, **90**, 7168–7171.
  128. K. M. Huber, M. S. Kayser and M. F. Bear, *Science*, 2000, **288**, 1254–1257.
  129. K. M. Huber, S. M. Gallagher, S. T. Warren and M. F. Bear, *Proc. Natl Acad. Sci. USA*, 2002, **99**, 7746–7750.
  130. M. F. Bear, K. M. Huber and S. T. Warren, *Trends Neurosci.*, 2004, **27**, 370–377.
  131. G. Dolen, E. Osterweil, B. S. Rao, G. B. Smith, B. D. Auerbach, S. Chattarji and M. F. Bear, *Neuron*, 2007, **56**, 955–962.
  132. S. Jacquemont, A. Curie, V. des Portes, M. G. Torrioli, E. Berry-Kravis, R. J. Hagerman, F. J. Ramos, K. Cornish, Y. He, C. Paulding, G. Neri, F. Chen, N. Hadjikhani, D. Martinet, J. Meyer, J. S. Beckmann, K. Delange, A. Brun, G. Bussy, F. Gasparini, T. Hilse, A. Floesser, J. Branson, G. Bilbe, D. Johns and B. Gomez-Mancilla, *Sci. Transl. Med.*, 2011, **3**(64), ra1–9.
  133. J. L. Rubenstein and M. M. Merzenich, *Genes Brain Behav.*, 2003, **2**, 255–267.
  134. J. L. Olmos-Serrano, S. Paluszkiwicz, B. S. Martin, W. E. Kaufmann and J. G. Corbin, and M. M. Huntsman, *J. Neurosci.*, 2010, **30**, 9929–9938.
  135. C. L. Gatto and K. Broadie, *Front Synaptic Neurosci.*, 2010, **2**, 4.
  136. R. Vigot, S. Barbieri, H. Brauner-Osborne, R. Turecek, R. Shigemoto, Y. P. Zhang, R. Lujan, L. H. Jacobson, B. Biermann, J. M. Fritschy, C. M. Vacher, M. Muller, G. Sansig, N. Guetg, J. F. Cryan, K. Kaupmann, M. Gassmann, T. G. Oertner and B. Bettler, *Neuron*, 2006, **50**, 589–601.
  137. B. H. Gahwiler and D. A. Brown, *Proc. Natl Acad. Sci., USA*, 1985, **82**, 1558–1562.
  138. J. R. Chalifoux and A. G. Carter, *Neuron*, **66**, 101–113.
  139. L. Lubsch, R. Habersang, M. Haase and S. Luedtke, *J. Child Neurol.*, 2006, **21**, 1090–1092.
  140. R. S. Hammond, C. S. Henderson, M. Shumway, C. Bryncka, R. Rush and A. Healy, *Society for Neuroscience*, 774.05, 2011.

141. L. K. K. Pacey, S. P. Heximer and D. R. Hampson, *Mol. Pharmacol.*, 2009, **76**, 18–24.
142. R. J. Hagerman, B. Rathmell, L. Wang, R. Carpenter, M. Bear, P. P. Wang and E. Berry-Kravis, in *Pediatric Academic Societies Annual Meeting*, Denver, 2011.
143. P. Wang, C. A. Erickson, L. Ginsberg, B. Rathmell, M. Cherubini, P. Zarevics and B. King, in *International Meeting for Autism Research*, San Diego, 2011.
144. A. J. Guastella, S. L. Einfeld, K. M. Gray, N. J. Rinehart, B. J. Tonge, T. J. Lambert and I. B. Hickie, *Biol. Psychiatry*, 2009, **67**, 692–694.
145. E. Andari, J. R. Duhamel, T. Zalla, E. Herbrecht, M. Leboyer and A. Sirigu, *Proc. Natl Acad. Sci. USA*, 2010, **107**, 4389–4394.
146. M. Sala, D. Braidà, D. Lentini, M. Busnelli, E. Bulgheroni, V. Capurro, A. Finardi, A. Donzelli, L. Pattini, T. Rubino, D. Parolaro, K. Nishimori, M. Parenti and B. Chini, *Biol. Psychiatry*, 2011, **69**(9), 875.
147. J. J. Bissler, F. X. McCormack, L. R. Young, J. M. Elwing, G. Chuck, J. M. Leonard, V. J. Schmithorst, T. Laor, A. S. Brody, J. Bean, S. Salisbury and D. N. Franz, *N. Engl. J. Med.*, 2008, **358**(2), 140–151.
148. D. M. Davies, S. R. Johnson, A. E. Tattersfield, J. C. Kingswood, J. A. Cox, D. L. McCartney, T. Doyle and F. Elmslie, A. Saggat, P. J. de Vries and J. R. Sampson, *N. Engl. J. Med.*, 2008, **358**, 200–203.
149. D. A. Krueger, M. M. Care, K. Holland, K. Agricola, C. Tudor, P. Mangeshkar, K. A. Wilson, A. Byars, T. Sahmoud and D. N. Franz, *N. Engl. J. Med.*, 2010, **363**, 1801–1811.
150. S. E. Levy, D. S. Mandell and R. T. Schultz, *Lancet*, 2009, **374**, 1627–1638.
151. S. J. Spence and A. Thurm, *Lancet*, 2010, **375**, 2124–2125.
152. G. Dawson, S. Rogers, J. Munson, M. Smith, J. Winter, J. Greenson, A. Donaldson and J. Varley, *Pediatrics*, 2010, **125**, e17–e23.
153. K. Gotham, A. Pickles and C. Lord, *J. Autism Dev. Disord.*, 2009, **39**, 693–705.
154. I. L. Cohen, T. R. Gomez, M. G. Gonzalez, E. M. Lennon, B. Z. Karmel and J. M. Gardner, *J. Autism Dev. Disord.*, 2010, **40**, 246–254.
155. M. G. Aman, N. N. Singh, A. W. Stewart and C. J. Field, *Am. J. Ment. Defic.*, 1985, **89**, 492–502.
156. M. L. McPheeters, Z. Warren, N. Sathe, J. L. Bruzek, S. Krishnaswami, R. N. Jerome and J. Veenstra-Vanderweele, *Pediatrics*, 2011, **127**, e1312–e1321.
157. E. Schwarz, P. C. Guest, H. Rahmoune, L. W. Harris, L. Wang, F. M. Leweke, M. Rothermundt, B. Bogerts, D. Koethe, L. Kranaster, P. Ohrmann, T. Suslow, G. McAllister, M. Spain, A. Barnes, N. J. van Beveren, S. Baron-Cohen, J. Steiner, F. E. Torrey, R. H. Yolken and S. Bahn, *Mol. Psychiatry*, 2012, **17**(5), 494.

## CHAPTER 16

# *Recent Chronology of Orexin Pharmacology and Its Potential as a Treatment for Primary Insomnia*

CHRISTOPHER J. WINROW,<sup>a</sup> ANTHONY L. GOTTER,<sup>a</sup>  
PAUL J. COLEMAN,<sup>b</sup> RICHARD HARGREAVES<sup>a</sup>  
AND JOHN J. RINGER<sup>\*a</sup>

<sup>a</sup> Merck Research Laboratories, Neuroscience Department, 770 Sumneytown Pike, West Point, PA 19486, USA; <sup>b</sup> Merck Research Laboratories, Medicinal Chemistry Department, 770 Sumneytown Pike, West Point, PA 19486, USA

\*Email: john\_renger@merck.com

## 16.1 Introduction

Narcolepsy is a disorder associated with excessive daytime sleepiness and an inability to maintain vigilance states. Identification of the orexin 2 receptor (OX<sub>2</sub>R) gene as the locus responsible for canine narcolepsy<sup>1</sup> was not only a landmark discovery for researchers investigating the etiology of this disorder, but also marked the beginning of investigations into the function of orexin in sleep and its control of vigilance state. Nearly simultaneous was the description of the narcoleptic phenotype of mice harbouring a targeted disruption of the prepro-orexin gene<sup>2</sup> and shortly thereafter by reports of genetic and

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

auto-immune-induced deficiencies in orexin signalling in human patients.<sup>3,4</sup> Orexin is both necessary for the normal control of vigilance state, and sufficient to induce arousal.<sup>5</sup> Orexin secreting neurons have daily oscillations in activity that give rise to elevated peptide release during waking hours and minimal levels during the normal inactive period.<sup>6,7</sup> As such, the actions of reagents and therapeutic compounds antagonizing orexin signalling are expected to reduce hyperarousal, providing effects similar to those observed during the normal sleep period. These discoveries provided the basis for ongoing studies utilizing genetically engineered mouse and rat models both to mimic the disease and to probe the mechanism of orexin signalling components in sleep and narcolepsy/cataplexy. Importantly, they also provided genetic proof-of-concept for pharmacological therapeutics targeting orexin receptors for the treatment of insomnia.

Orexin peptides and receptors are highly conserved across species, exhibiting >90% amino acid homology between mice and humans. The 131 amino acid (a.a.) human prepro-orexin peptide is cleaved to form orexin A (OX-A, 33 a.a.) and orexin B (OX-B, 28 a.a.), which signal through activation of two G protein coupled receptors, orexin 1 and orexin 2 receptors (OX<sub>1</sub>R and OX<sub>2</sub>R, respectively). OX-A binds and activates OX<sub>1</sub>R and OX<sub>2</sub>R with equal affinity, whereas OX-B shows ~10-fold higher affinity for OX<sub>2</sub>R. Distribution of orexinergic neurons is localized to approximately 50,000 cells within the lateral and medial hypothalamus that project widely throughout the brain.<sup>8,9</sup> Receptor subtypes show overlapping and distinct expression, with both forms found in dorsal raphe (DR) nuclei, the ventral tegmental area (VTA), pedunclopontine (PPT) and laterodorsal tegmental nuclei (LDT). OX<sub>2</sub>R is enriched in histaminergic neurons of tuberomammillary nuclei (TMN) while OX<sub>1</sub>R expression appears restricted to regions including the locus coeruleus.<sup>10</sup> Recently, we have demonstrated that structurally distinct orexin receptor antagonists maintain comparable *in vitro* potency towards orexin receptors from mice, rats, rabbits, dogs, monkeys and humans.<sup>11,12</sup> This maintenance of potency and affinity can be correlated with the high degree of structural and functional conservation between orexin receptors from diverse species, and correlates with translational efficacy of orexin receptor antagonists for promoting sleep in rodents, dogs, monkeys and humans.

## 16.2 Current Understanding of Orexin Biology – From Genetics to Pharmacology

### 16.2.1 Genetic Dissection of Orexin Function

Genetic evidence for the control of vigilance state by orexin signalling was demonstrated by the concurrent discoveries of a disruption of the OX<sub>2</sub>R gene responsible for genetically transmitted canine narcolepsy<sup>1</sup> and the description of the narcoleptic phenotype associated with targeted disruption of the mouse *prepro-orexin* gene (OX knockouts).<sup>2</sup> In both cases, the phenotype of



these animals included hypersomnolence and sleep architecture changes including decreased latency to rapid eye movement (REM) sleep along with fragmented non-REM (NREM) sleep occurring with unusually rapid transitions. Hallmark cataplectic symptoms were observed in both species including muscular atonia manifested as abrupt behavioural arrest accompanied by what appeared to be intrusion of REM into the normal active phase, and sleep architecture characterized by Wake  $\rightarrow$  REM transitions replacing the typical Wake  $\rightarrow$  NREM  $\rightarrow$  REM progression.<sup>1,2,13</sup> Linkage analysis and positional cloning identified OX<sub>2</sub>R truncation mutations in Doberman Pinschers and Labrador Retrievers resulting in narcolepsy/cataplexy symptoms transmitted in an autosomal recessive manner with complete penetrance,<sup>1</sup> findings enabling the later identification of point mutation in the Dachshund family with genetic narcolepsy.<sup>14</sup> Transgenic mouse and rat models in which orexin secreting neurons have been ablated through the expression of a cytotoxic Ataxin-3 gene under the control of an orexin promoter have also been developed. These animals (OX/Atx mice), which more closely mimic the majority of human narcoleptic patients harbouring auto-immune-mediated loss of orexin neurons, exhibit a phenotype even more pervasive than OX knockouts.<sup>15–17</sup> Reproducibility of the narcolepsy/cataplexy phenotype of both OX/Atx transgenic animals and OX knockouts has provided a standard set of criteria for cataplexy against which all other genetic mouse models have been compared.<sup>2,18,19</sup>

### 16.2.1.1 OX Knockouts and OX/Atx Transgenics

Genetic models have provided insights into the mechanisms through which orexins control arousal, and how disruption of the associated signalling components leads to dysregulation of sleep/wake control. In OX knockouts, loss of OX-A and OX-B peptides leads to vigilance state instability, while the circadian control of sleep/wake timing and homeostatic responses to sleep deprivation appears unaffected.<sup>20</sup> In the absence of daily light cues, OX knockouts exhibit a normal circadian period and the amplitude of daily rhythms in locomotor activity and sleep/wake are indistinguishable from wild types, and recovery from sleep deprivation produces no difference in REM or NREM time between genotypes. OX knockouts, however, display unusually rapid sleep state transitions marked by very short bouts of wake, NREM and REM, even under normal conditions.<sup>20,21</sup> In addition to cataplexy-like symptoms punctuated by active phase behavioural arrest episodes in mutants,<sup>2</sup> high-resolution qEEG and polysomnographic analysis indicates that OX knockouts exhibit greater overlap in spectral signatures between wake and REM sleep EEG than their wild-type counterparts, suggesting a blurring of the boundaries between vigilance states.<sup>22</sup> Consistent with this idea, mutants also demonstrate more transitions between states occurring more rapidly and in an unregulated manner; the normal Wake  $\rightarrow$  NREM  $\rightarrow$  REM progression is often replaced by transitions involving direct Wake  $\rightarrow$  REM transitions in OX

knockouts, demonstrating that boundaries between vigilance states are less clearly defined in these animals.<sup>22</sup>

Transgenic mice and rats lacking orexin-containing neurons (OX/Atx) exhibit symptoms similar to mice with targeted disruption of the *prepro-orexin* gene.<sup>15–17</sup> Although circadian timing is also unaffected in OX/Atx animals, REM dysregulation is more pervasive than that seen in their OX knockout counterparts, the former exhibiting an even greater number of state transitions and time spent in REM sleep during the animals' subjective active phase.<sup>21</sup> These results suggest that other factors released from orexin-containing neurons contribute to sleep architecture in addition to OX-A and OX-B, and may include glutamate and dynorphin, as these transmitters are known to be released by orexin-containing neurons.<sup>21,23</sup> Recent *in vitro* evidence suggests that dynorphin modulates the sensitization and firing rate of orexin-sensitive neurons, resulting in a delay in orexin-induced arousal at the sleep/wake transition.<sup>24</sup>

### 16.2.1.2 *OX<sub>2</sub>R Mutations*

Similar to ligand mutant animals, targeted mutagenesis of OX<sub>2</sub>R results in narcolepsy-like symptoms including hypersomnolence ("sleep attacks"), fragmented wakefulness and NREM sleep, increased active phase NREM sleep and rapid behavioural state transitions.<sup>25</sup> In wild-type animals, icv administered OX-A or OX-B induces arousal, promotes locomotor activity and increases core temperature and wakefulness. In OX<sub>2</sub>R mutant mice, however, OX-A or OX-B-induced arousal is largely absent, indicating that the wake promoting effects of orexin are primarily through OX<sub>2</sub>R.<sup>13,26–28</sup> Similarly, in narcoleptic dogs harbouring truncation mutations in the gene for OX<sub>2</sub>R, exogenously applied OX-A is completely ineffective in rescuing the cataplectic or hypersomnolence phenotype of these animals.<sup>29</sup> Because orexin-containing neurons themselves express OX<sub>2</sub>R and respond positively to orexin peptides, an OX<sub>2</sub>R-mediated positive feedback mechanism may further accentuate the role of this receptor.<sup>30</sup> In support of this idea, whole cell patch recording of hypothalamic slice recordings, OX-B effectively induced depolarization of orexin neurons from both wild-type and OX<sub>1</sub>R knockout animals, but failed to do so in tissue from OX<sub>2</sub>R knockouts.<sup>31</sup>

Much of the arousal mediated through OX<sub>2</sub>R is thought to occur through histaminergic neurons of the tuberomammillary nuclei (TMN).<sup>32,33</sup> In TMN containing brain slices OX-A typically induces transient increases in intracellular Ca<sup>2+</sup>, but in preparations from OX<sub>2</sub>R knockouts, this response is absent.<sup>25</sup> Further evidence for the role of histamine in OX-A-induced arousal is the observation that histamine H<sub>1</sub> receptor blockade with pyrilamine blocks the wake-promoting effects of icv administered OX-A.<sup>34</sup> The somnolence-promoting effects of OX<sub>2</sub>R and dual OX<sub>1</sub>R and OX<sub>2</sub>R antagonism are also associated with attenuated histamine levels in lateral hypothalamus as evaluated by microdialysis,<sup>35</sup> substantiating the role of OX<sub>2</sub>R in mediating orexin and histamine-induced arousal.

### 16.2.1.3 *OX<sub>1</sub>R Mutations*

OX<sub>1</sub>R knockouts reportedly exhibit normal sleep architecture with mild fragmentation (abstract cited in ref. 25). Nevertheless, the role of OX<sub>1</sub>R in arousal and vigilance state has been inferred by comparing the phenotypes of OX knockouts with that of OX<sub>2</sub>R mutants. OX<sub>2</sub>R knockouts lack the full narcolepsy/cataplexy phenotype of OX knockouts including only limited Wake → REM transitions and the behavioural arrests that are displayed are more gradual than the abrupt atonia observed in OX knockouts, suggesting that OX<sub>1</sub>R plays a role in controlling vigilance state transitions and atonia associated with active phase cataplectic episodes.<sup>25</sup> OX<sub>1</sub>R, but not OX<sub>2</sub>R is expressed in noradrenergic neurons of the locus coeruleus (LC), which are typically active during wake and quiescent during REM sleep.<sup>36,37</sup> LC neurons become active in response to local administration of OX-A, but not following a treatment with OX-B. Similarly, OX-A and not OX-B application to the LC promotes wakefulness and suppresses REM and SWS.<sup>36</sup> Conversely, pre-dosing of SB-334867, an OX<sub>1</sub>R antagonist, attenuates the REM suppressing effects of OX-A administration, while SB-334867 alone has little effect when applied at inactive phase onset, a time when endogenous orexin levels are minimal.<sup>38</sup> Still another study suggests general OX<sub>1</sub>R antagonism has effects on sleep and neurotransmitter release distinct from OX<sub>2</sub>R. SB-408124, an OX<sub>1</sub>R antagonist, attenuates the somnolence promoting effects of OX<sub>2</sub>R antagonism, and by itself induces extra-cellular dopamine levels in lateral hypothalamus while leaving histamine levels unchanged, suggesting a potential mechanism for countering OX<sub>2</sub>R activity.<sup>35</sup> Further study will determine the role of OX<sub>1</sub>R in orexin regulation of sleep/wake and its contribution to the differential phenotypes exhibited by animals in which orexin neurons are ablated and the *prepro-orexin* gene is disrupted by targeted mutagenesis.

## 16.2.2 Orexin Ligands Promote Wakefulness and Arousal

Endogenous orexin levels fluctuate over the course of the day with levels building during the active period and reaching their nadir during the normal sleep phase,<sup>6,7</sup> providing a marker for wakefulness and driving arousal. Exogenously applied OX-A increases locomotor activity and grooming, and promotes wakefulness at the expense of NREM and REM sleep. As might be predicted for an arousal-promoting peptide, these effects are much more pronounced during the animals' subjective inactive phase, a time at which endogenous orexin levels are at their lowest.<sup>26,27</sup> In mice with genetic ablation of orexin-secreting neurons, exogenously applied OX-A promotes arousal to levels exceeding that seen in wild-type animals when treated identically, not only indicating downstream orexin signalling components including OXRs remain in these mutants and are up-regulated, but that the neuropeptide alone is sufficient as a wakefulness signal.<sup>39</sup> Similarly, OX-A rescues the phenotype of narcoleptic dogs harbouring a mutation in the gene encoding the prepro-orexin ligand precursor, but in dogs with mutations in the gene for OX<sub>2</sub>R it has no

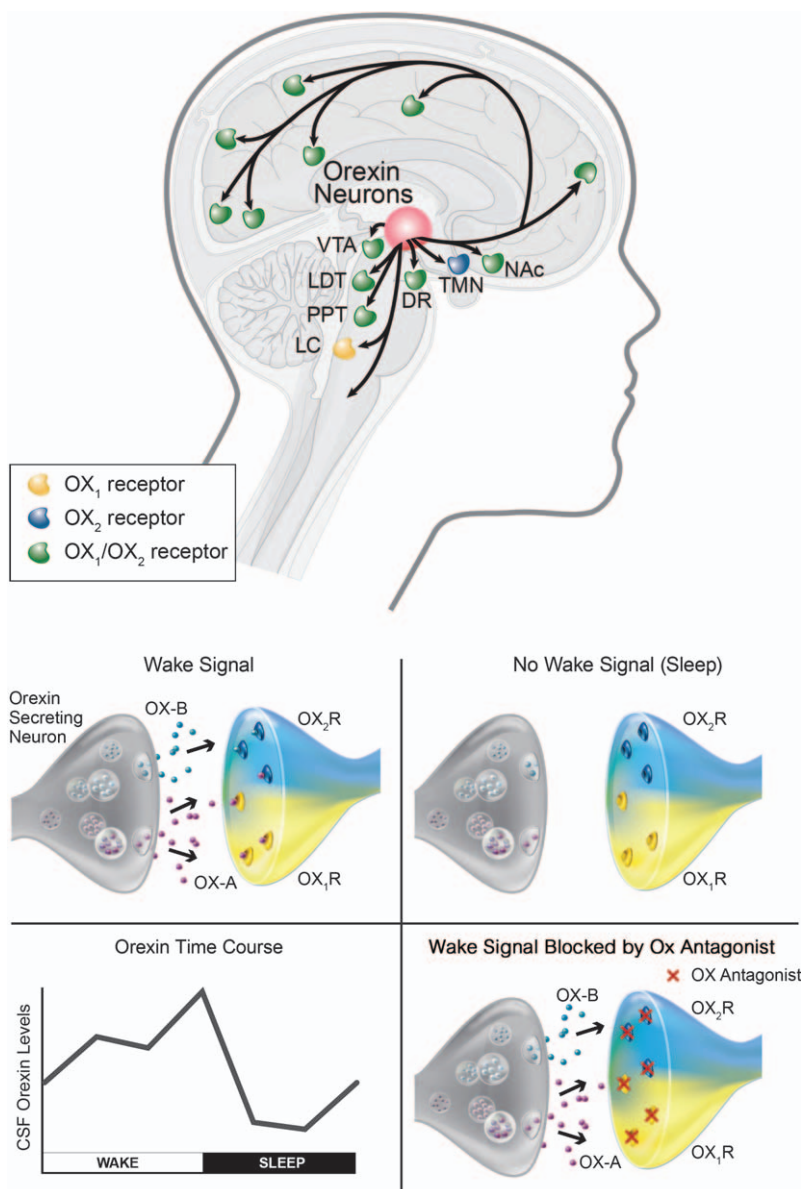
effect on the cataplexy or hypersomnolence phenotype.<sup>29</sup> Activation of orexin-secreting neurons by optigenetic means also increases waking, demonstrating that endogenous orexin is sufficient to drive wakefulness.<sup>40,41</sup> Taken together with the observed hypersomnolence phenotype associated with mutations in orexin signalling components, these results indicate that orexin is both necessary and sufficient for normal sleep/wake regulation and arousal.

### 16.2.3 Orexin-mediated Pathways Controlling Sleep Architecture

Afferent connections between orexin neurons in the lateral hypothalamus (LH) and arousal regulating regions including tuberomammillary nuclei (TMN), dorsal raphe nuclei (DRN), locus coeruleus (LC), ventral tegmental area (VTA), cortex (Ctx) and supra-chiasmatic nuclei (SCN) are conserved across species. Orexin neurons located within the hypothalamus receive inputs from sensory neurons responsive to light, energy levels, homeostatic sleep need, visceral sensory signals and neurons within the limbic cortex (Figure 16.1). The integration of these inputs results in modulation of orexin levels in response to circadian signals and light cues, as evidenced by alterations of orexinergic activity and orexin levels in response to changes in external circadian cues. As such, orexin signalling has been proposed as a “sleep-switch” based on reciprocal innervations between sleep/wake regions in the CNS.<sup>42,43</sup> This switch is hypothesized to govern arousal state transitions into wake and serve to initiate and maintain wakefulness based on appropriate external cues, including light/dark phase. Interestingly, this relationship is maintained in diurnal, nocturnal and polyphasic animals. For example, increased orexin signalling is observed preceding light onset in diurnal animals, whereas increased orexin neuron activity occurs just prior to dark phase in nocturnal animals.<sup>5</sup> Orexin cell loss not only diminishes arousal, but also disorganizes sleep architecture. Vigilance state disorganization is associated with orexin cell ablation in both pre-clinical animal models and in human narcoleptic patients, and is manifested as REM sleep intrusions into wakefulness and excessive daytime sleepiness (EDS).<sup>2,44</sup> Evidence now indicates that orexin signalling modulates sleep/wake through two different mechanisms – cortical arousal predominantly mediated by  $OX_2R$  signalling through the TMN, while the gating between vigilance states is controlled by both  $OX_2$  and  $OX_1$  receptor activity in brainstem regions.

A major pathway mediating the arousal-promoting effects of orexin is conveyed through  $OX_2R$  activity on histaminergic neurons of the TMN. The predominant role of  $OX_2R$  in orexin-mediated arousal has been demonstrated both genetically and pharmacologically. The hypersomnolence observed in  $OX_2R$  knockouts is similar to that observed in  $OX$  mutants and most, if not all, of the wake-promoting effects of exogenously administered  $OX-A$  are largely absent in  $OX_2R$  mutant animals.<sup>25,29,45</sup> Additionally, icv administration of  $OX-B$  and [ala11]- $OXB$ , which are 10- and 120-fold selective for  $OX_2R$ , respectively, are capable of inducing arousal and suppressing NREM and

REM sleep to levels similar that of the response to OX-A.<sup>26,27,46</sup> The TMN appears to be a main site of action, since it is one of the few brain regions in which OX<sub>2</sub>R is preferentially expressed over OX<sub>1</sub>R.<sup>47,48</sup> Further, adenoviral-mediated focal expression of OX<sub>2</sub>R within the TMN of animals that are otherwise mutant for this receptor rescues the arousal deficits of these narcoleptic OX<sub>2</sub>R knockouts. The sleep fragmentation phenotype of these animals,



however, remained unaffected, indicating that  $OX_2R$  activity in the TMN has a selective role in arousal, while the control of vigilance state gating resides elsewhere.<sup>49</sup> The importance of  $OX_2R$ -controlled histamine release is supported by evidence showing that the wake-promoting effects of exogenously applied orexin peptides are suppressed by the histamine synthesis inhibitor, alpha-fluoromethylhistidine (FMH),<sup>50</sup> the Histamine 1 receptor antagonist pyrilamine<sup>34</sup> or by genetic ablation of H1 receptor.<sup>32</sup> The somnolence-promoting effects of selective  $OX_2R$  antagonism is also associated with attenuated extracellular histamine levels in the lateral hypothalamus.<sup>35</sup>

Orexin also has important influences on brainstem nuclei controlling vigilance state and sleep architecture. Orexin neurons from the hypothalamus project to these regions, and both OXRs show expression in the VTA and DRN, as well as laterodorsal and pedunculopontine nuclei (LDT, PPT), while  $OX_1R$  expression appears preferentially expressed in the LC.<sup>47,48</sup> Together these nuclei are largely responsible for controlling thalamocortical relay activity responsible for EEG tone, vigilance state, REM-associated muscular atonia and ultimately sleep architecture, and can be generally classified as “REM-on” (LDT, PPT) or “REM-off” (LC, DR).<sup>5</sup> OX-A injected directly into the LC increases action potential firing and promotes wakefulness by 70% while decreasing NREM by 48% and fully suppressing REM sleep.<sup>36,51</sup> OX-A injection into the LDT of cats also increases wake and suppresses REM sleep<sup>52</sup> while producing long-lasting excitation of these cholinergic cells.<sup>53</sup> Together these results suggest that  $OX_1R$  activity in arousal producing brainstem nuclei contributes to the wake-promoting and REM-suppressing effects of these brainstem nuclei.

**Figure 16.1** Orexin efferent pathways mediating wakefulness. Upper panel: orexinergic neuron projections are depicted by black arrows, brain nuclei exhibiting preferential  $OX_1R$  expression in yellow, preferential  $OX_2R$  expression in blue and both  $OX_1$  and  $OX_2$  receptor expression in green. TMN, histaminergic tuberomammillary nuclei; VTA, ventral tegmental area; LDT and PPT, laterodorsal and pedunculopontine tegmental nuclei; DR, dorsal raphe nuclei; LC, locus coeruleus; NAc, nucleus accumbens. Bottom panels: orexin peptide-induced wakefulness and orexin receptor antagonist-induced block of wakefulness.  $OX_1$  receptors are depicted in yellow,  $OX_2$  receptors in blue. OX-A has activity for both  $OX_1R$  and  $OX_2R$  with  $IC_{50}$ s of 20 and 38 nM, respectively, while OX-B is more selective for  $OX_2R$  ( $IC_{50}$  of 36 nM versus 420 nM for  $OX_1R$ ).<sup>110</sup> Orexin-mediated signalling is associated with wakefulness (top left) and falls silent during sleep (top right). Orexin antagonists block the orexin wakefulness signal to promote sleep. The bottom left panel depicts representative levels of orexin found in CSF based on Taheri *et al.* (2000), Zeitzer *et al.* (2003) and unpublished observations, in which levels of orexin peptides build over the course of the active period in response to orexinergic signalling and reach a nadir during the inactive period. Note that similar patterns are observed in nocturnal animals under reversed light/dark conditions.

Graphic design by Jill Williams and Lynn Schoeninger, Visual Communications Department, Merck Research Laboratories.



The role of OX<sub>1</sub>R in brainstem nuclei, particularly in neurons of the LC, has emerged as an important factor in the regulation of vigilance state gating. Increased firing of LC neurons along with REM-suppressing effects of direct injection of OX-A are blocked by pre-incubation of the micro-injection site with an OX<sub>1</sub>R neutralizing antibody.<sup>36</sup> Knock down of OX<sub>1</sub>R in LC was observed to be associated with inappropriate increases in REM sleep during the active period of rats for up to 4 days following treatment, a time course coincident with reduced OX<sub>1</sub>R mRNA levels. Remarkably, neither wakefulness, NREM sleep nor qEEG power spectra of treated animals are affected, indicating that this effect is specific for vigilance state gating and not arousal.<sup>54</sup> Conversely, brainstem micro-injection of OX-A during wakefulness in cats results in a decrease in REM time and frequency.<sup>55</sup> Muscarinic acetylcholine (ACh)-mediated signalling from pontine neurons may influence this signalling since pharmacological increases in ACh activity are associated with increases in cataplexy-associated behavioural arrest number without affecting mean arrest time in narcoleptic OX<sub>1/2</sub>R double knockout animals.<sup>56</sup> Still, the overall importance of OX<sub>1</sub>R in sleep architecture remains to be resolved given the mild, barely detectable phenotype of the constitutive mutant animals. The development of potent OX<sub>1</sub>R antagonists whose selectivity are improved over those currently available will be invaluable toward this end.

### 16.2.4 Modulation of Orexin Signalling

Modulation of orexin signalling occurs both locally within the lateral hypothalamus and post-synaptically on orexin-responsive cells to coordinate sleep architecture and to align the timing of sleep with physiological needs and environmental cues. Orexin cell firing is increased by direct ACh, Glu and ghrelin application and decreased by leptin, glucose, NE, 5-HT and GABA.<sup>57</sup> Orexin neurons also express adenosine A1 receptors. Adenosine increase is a putative physiologic generator of sleep pressure based on data showing extracellular adenosine levels increase as a function of time awake and during sleep deprivation. Application of the adenosine A1R antagonist, 1,3-dipropyl-8-phenylxanthine (DPX) into the lateral hypothalamus increases wake and suppresses both REM and NREM sleep for the first 3 hours after dosing.<sup>58</sup> Micro-infusion of the adenosine A2A agonist, CGS21680, into the ventral striatum both promotes somnolence and attenuates c-Fos production in orexin-producing cells. This suggests adenosine's sleep-promoting effect may be facilitated by reduced orexinergic cell firing and that orexin signalling is not necessarily the exclusive mechanism for adenosine-mediated sleep promotion.<sup>59</sup>

Melanin concentrating hormone (MCH)-producing cells are localized in the hypothalamus adjacent to orexin-producing neurons, and have patterns of activity distinct from their orexin-secreting neighbors.<sup>60</sup> Activity of each cell group assessed by c-Fos imaging finds MCH neurons to have an opposite pattern of activity to orexin neurons, with MCH c-Fos levels being elevated during sleep and orexin-induced c-fos levels high during wakefulness.<sup>61,62</sup>



GABAergic neurons have also been identified in the lateral hypothalamus, intermingled but distinct from both orexin- and MCH-secreting cells present there. Because these GABA-secreting neurons are specifically active during both REM and NREM sleep, they may provide a local inhibitory influence on orexin-secreting neurons whose activity is associated with arousal.<sup>63</sup> GABAergic influences on OX<sub>1</sub>R-mediated signalling also appear to occur within the pontine reticular formation, as bicuculine antagonism of GABA<sub>A</sub> receptor activity blocks OX-A-induced arousal.<sup>64</sup> Together these influences on orexin signalling modulate the timing and physiological requirements for arousal and vigilance state.

Orexin's effect on arousal also appears to be modulated by corticotropin releasing hormone (CRH) and dopamine. CRH signalling involved in stress responses represents another possible interaction with orexin, since elevated levels of the hormone are associated with arousal and icv administered CRH induces wakefulness and locomotor activity.<sup>65</sup> The interaction with CRH signalling appears to be upstream of orexin, since orexin-induced arousal persists in CRH receptor knockouts as well as in the presence of CRH receptor antagonists.<sup>66</sup> Evidence for the modulation of orexin signalling in arousal and psychiatric function by dopamine has also recently emerged.<sup>65,67</sup> In *prepro-orexin* knockout mice, pharmacological D<sub>1</sub> receptor activation decreases the prevalence of sleep attacks relative to wild-type animals. Conversely, the hypersomnolence of these animals is exacerbated with D<sub>1</sub> antagonism, while D<sub>2</sub> receptor modulation had little or no effect on arousal.<sup>68</sup> Cataplectic attacks, however, are affected by D<sub>2</sub> receptor activity; pharmacological D<sub>2</sub> activation and inhibition is associated with substantial increases and decreases, respectively, in behavioural arrest.<sup>68</sup> These studies further illustrate the existence of distinct pathways for arousal control and the regulation of vigilance state as well as the contributions of dopamine receptor subtypes in these processes. Together with the observation that OX<sub>1</sub>R antagonism has the potential to elevate prefrontal dopamine levels and attenuate OX<sub>2</sub>R antagonist-mediated somnolence,<sup>35</sup> this suggests that dopaminergic signalling through OX<sub>1</sub>R has the potential not only to modulate OX<sub>2</sub>R-mediated CNS arousal, but also to regulate gating through vigilance state transitions. Additional studies in these areas will certainly be informative.

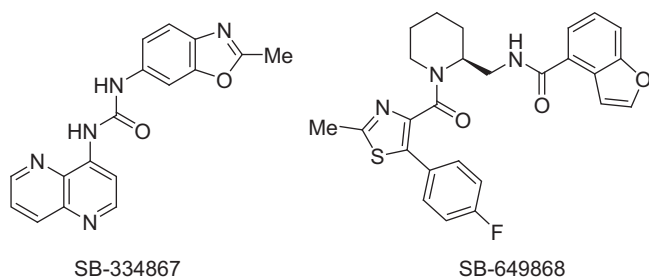
## 16.3 Identification of Orexin Receptor Antagonists

Efforts to synthesize orexin receptor antagonists began shortly after these G-protein coupled receptors were deorphanized in the late 1990s. Ligands to orexin receptors have been identified by a number of research groups using high-throughput screening to provide high-affinity chemical leads. Indeed, researchers at Actelion, SmithKline Beecham (now GlaxoSmithKline) and Merck initiated programs to identify and develop orexin receptor antagonists. Each of these efforts was rewarded by the identification of orexin receptor antagonists that have subsequently undergone clinical evaluation. Several other

laboratories including Johnson & Johnson, Roche and Sanofi-Aventis have since joined the pursuit of orexin antagonists.<sup>69</sup>

### 16.3.1 OX<sub>1</sub>R-selective Antagonism to Probe *in vivo* Receptor Function

Researchers at GSK were among the first to identify small-molecule orexin receptor antagonists with a series of patent applications describing a range of chemotypes. One of the earliest publications described a series of heterocyclic ureas including SB-334867 as OX<sub>1</sub>R-selective antagonists (Figure 16.2). SB-334867 was reported to be efficacious in an orexin-A-induced food intake study, and subsequent publications have described weight loss in rats and reductions of amphetamine sensitization following administration of SB-334867. This molecule has been utilized extensively as an OX<sub>1</sub>R-selective pharmacological probe. SB-334867 is a brain penetrant OX<sub>1</sub>R antagonist measureable in the CNS for up to 2 hours post dose. When administered IP at 10 and 30 mpk, SB-334867 reverses the reduction of REM sleep produced by a 10 µg icv injection of OX-A prior to the rat active period.<sup>38</sup> Evaluated alone, OX-A icv in this study does not modify wake or NREM in the first hour after injection and SB-334867 at these doses produced no reported effects on REM duration or latency. In a subsequent study, SB-334867 was dosed at 30 mg/kg IP, providing a significant decrease in wake and a significant increase in NREM sleep (no change in REM) as assessed in the first 4 hours after wake period dosing.<sup>70</sup> When SB-334867 was injected directly into the rat pontine reticular formation it blocked the wake-promoting effects of direct pontine application of OX-A.<sup>64</sup> Overall these data suggest that OX<sub>1</sub>R activity in the brainstem is important for wake promotion but thereafter the brainstem location of these receptors determines the effects of OX<sub>1</sub>R on specific states, a result consistent with our knowledge of brainstem control of sleep/wake and REM/NREM. However, some caution must be exercised in the interpretation of these and other studies utilizing SB-334867 as the selectivity of this compound for OX<sub>1</sub>R is only ~50-fold over that for OX<sub>2</sub>R,<sup>71,72</sup> such that *in vivo* dose response data are unobtainable without significant OX<sub>2</sub>R engagement at higher doses. Of additional concern are potential off-target effects of this compound.



**Figure 16.2** GlaxoSmithKline orexin antagonists.

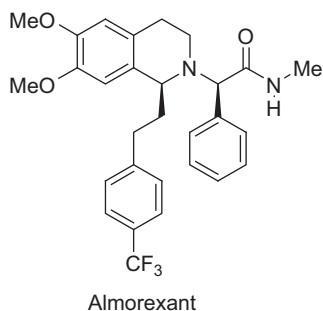
Evaluation of SB-334867 in a panel of 170 other enzymes, transporters and receptors revealed significant activity towards the adenosine<sub>A2A</sub> receptor ( $K_i = 0.67 \mu\text{M}$ ), 5-HT<sub>2C</sub> receptor ( $1.2 \mu\text{M}$ ), monoamine transporter ( $1.44 \mu\text{M}$ ), norepinephrine transporter ( $1.58 \mu\text{M}$ ), adenosine transporter ( $2.45 \mu\text{M}$ ), adenosine<sub>A3</sub> receptor ( $3 \mu\text{M}$ ) and 5-HT<sub>2B</sub> receptor ( $3.47 \mu\text{M}$ ) (unpublished observations).

### 16.3.2 Dual Orexin Receptor Antagonists Selectively Attenuate Arousal

In addition to efforts developing OX<sub>1</sub>R-selective ligands, researchers at GSK discovered a series of piperidine amide antagonists including SB-649868 as potent dual OX<sub>1</sub>R/OX<sub>2</sub>R receptor antagonists (DORAs) (see Figure 16.2). This molecule was reported to potently inhibit OX<sub>1</sub>R and OX<sub>2</sub>R and entered clinical trials in 2005. Preclinically, SB-649868 has been shown to be sleep-promoting in rodent and primate studies.<sup>73</sup> In 2007, GSK announced that SB-649868 had advanced to phase II clinical trials. In initial single rising dose studies, SB-649868 administered in doses ranging from 10 to 80 milligrams was well tolerated and exhibited proportional increases in exposure across the dose range. Plasma half-lives ranged from 4 to 7 hr with rapid absorption ( $T_{\text{max}} = 1\text{--}3 \text{ hr}$ ). SB-649868 was administered in three cohorts of healthy volunteers to assess drug effects on safety, pharmacokinetics and EEG measures with daytime dosing, and to assess objective measures of sleep by polysomnography (PSG) following evening dosing.<sup>74</sup>

Following administration of SB-649868, drug effects on sleep induction and maintenance were assessed in healthy volunteers at 30 and 60 milligrams. Statistically significant improvements on Total Sleep Time (TST) *versus* placebo were recorded at 30 and 60 mg. SB-649868 also reduced Latency to Persistent Sleep (LPS) and reduced Wake After Sleep Onset (WASO) at both doses. Neither dose produced cognitive impairment as measured by a digit symbol substitution test (DSST) in the morning following evening drug administration. Clinical studies revealed that SB-649868 increased exposure of co-administered simvastatin in a drug–drug interaction study. With a 30 mg dose of SB-649868, total plasma exposure of co-administered simvastatin increased greater than 6-fold after 15 consecutive days of co-dosing. Consistent with these observations, SB-649868 was reported to be a potent inhibitor of CYP3A4 *in vitro*.<sup>74</sup> SB-649868 was placed on clinical hold in late 2007 from phase II studies due to the emergence of a reported preclinical toxicity. Subsequently, GlaxoSmithKline entered into collaborative agreement with Actelion in 2008 to co-develop Almorexant (*vide infra*) and other potential back-up compounds (Figure 16.3).

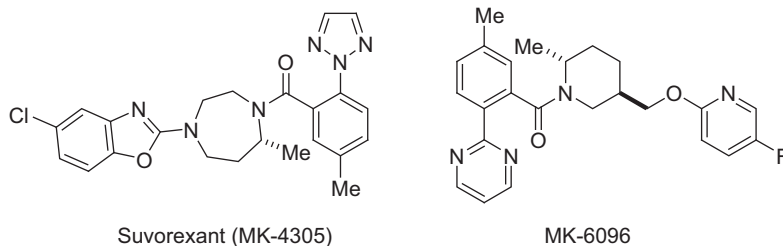
Efforts at Actelion Pharmaceuticals have produced multiple classes of orexin receptor antagonists including selective OX<sub>1</sub>R, OX<sub>2</sub>R and dual antagonists beginning as early as 2001. Significant effort has been invested in the tetrahydroisoquinoline (THIQ) series derived from a high-throughput screening



**Figure 16.3** Structure of Almorexant.

lead that was further optimized by combinatorial medicinal chemistry approaches. These efforts resulted in the discovery of Almorexant, a potent dual orexin receptor antagonist (Figure 16.3). When administered orally at 10–300 mg/kg, Almorexant dose-dependently increased both REM and NREM sleep in Wistar rats. These sleep effects were seen within 1 hour of dosing with effects lasting out to 12 hours at the highest dose. Effects of Almorexant on dog sleep, as measured by video recordings of mobility, were significant starting at 100 mg/kg in dogs when dosed in the active period. Later the same group found that 5 days of consecutive administration of a “pharmacological dose” produced consistent sleep effects across all dosing days without tachyphylaxis. Withdrawal of daily dosing did not produce residual or rebound sleep effects in rats or dogs.<sup>75</sup> In rats and dogs no evidence of cataplexy was seen at any dose, with only a 100 mpk dose significantly reducing dog mobility.<sup>76</sup> Interestingly, 30 mg/kg, but not 6 mg/kg, Almorexant promoted significantly more time in sparrow sleep postures (no EEG was recorded) suggesting birds respond to orexin antagonists like mammals.<sup>77</sup>

In a human ascending dose study (5–1,000 mg) of Almorexant oral administration produced dose responsive increases in plasma levels and elimination half-life ranging from 13.1 to 19.0 hours after wake period dosing to healthy males.<sup>78</sup> Peak plasma concentrations increased with rising doses (range 1.7–291 ng/ml) but total plasma exposure (AUCs) increased less than dose-proportionately (362–1,910 ng·hr/ml). Both self-reported and objective adverse endpoints were well tolerated with somnolence, dizziness, disturbed attention and fatigue reported above 200 mg. No cataplexy-related side-effects were seen and motor control problems in body sway and smooth pursuit eye movements were generally less than those produced by the clinical dose of Zolpidem (10 mg). PK/PD modelling of alertness effects suggested 500 mg Almorexant to be roughly equal to 10 mg Zolpidem. Specifics regarding the sleep effects of Almorexant on human sleep are emerging in the literature but Brisbare-Roch *et al.*<sup>76</sup> reported that doses above 200 mg produced dose-dependent reductions in latency to sleep onset, latency to NREM, increased sleep efficiency (% time asleep), increased total sleep time and increased total NREM time. Effects were reported to have ceased by 6.5 hours after dosing



**Figure 16.4** Merck orexin antagonists.

except for the 1,000 mg dose. Phase III clinical development of Almorexant was halted in January 2009 by Glaxo and Actelion due to an undisclosed human tolerability issue. Both companies remain active in the discovery of new orexin antagonists.

Merck has developed a diverse portfolio of orexin receptor antagonists in several structural classes.<sup>79</sup> Discovery efforts at Merck began in the late 1990s at the Banyu Tsukuba Research Institute. These early efforts led to the discovery of tetrahydroisoquinoline analogues that were  $OX_2R$ -selective ligands. After completing a screening campaign to identify new leads, efforts at Merck were subsequently broadened to the proline bis-amide series as well as the *N,N*-disubstituted-1,4-diazepane series. Diazepane lead molecules were discovered to have good affinity for both  $OX_1R$  and  $OX_2R$ . Lead optimization efforts generated orexin antagonists with improved potency and more favourable physicochemical properties. Early lead molecules in this effort suffered from poor preclinical pharmacokinetics and extensive hepatic metabolism. In addition to high rates of hepatic clearance, early molecules from this campaign produced reactive metabolites. Subsequent mechanistic studies identified sites of metabolism and defined routes of clearance. 5-Methyl substitution on the diazepane core was discovered to improve significantly potency and pharmacokinetics. Further structural modifications produced molecules with reduced bioactivation liability. From these efforts, Merck disclosed suvorexant (MK-4305; Figure 16.4) as a potent and selective dual orexin receptor antagonist with excellent activity in cell-based assays ( $OX_1R$   $IC_{50}$  = 50 nM,  $OX_2R$   $IC_{50}$  = 56 nM) and >6,000-fold selectivity against a panel of 170 receptors and enzymes. Suvorexant is orally bioavailable, has good brain penetrance and demonstrates orexin receptor occupancy in rat brain. In rodent sleep studies, suvorexant dose-dependently reduced active wake and increased REM and NREM sleep when administered orally at 10, 30 and 100 mg/kg.<sup>11,80</sup> Based on these favourable preclinical data, suvorexant was advanced into clinical development.

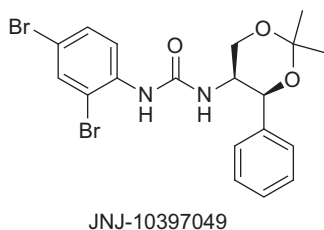
In phase I studies, suvorexant was well tolerated with peak plasma levels achieved at 1.5–4 hr and a terminal plasma half-life of 8–14 hr.<sup>81</sup> In healthy volunteers, dose-dependent observations of somnolence were evident. When administered in the evening, next day residual sedation was not observed with suvorexant at 10 and 50 mg doses while these same doses provided

dose-dependent increases in overall sleep efficiency and reductions in WASO and LPS. Results from the phase IIb study demonstrated that suvorexant was superior to placebo in improving sleep efficiency on the first night of treatment as well as at the end of four weeks in patients with primary insomnia.<sup>81</sup> These improvements in sleep efficiency were noted at all doses (10, 20, 40 and 80 mg). Suvorexant also showed improvements in the secondary endpoints of reduced WASO at all doses and reduced LPS at a dose of 80 mg. In 2010, Merck announced that MK-4305 had entered into phase III development. Suvorexant remains the most advanced orexin antagonist in active clinical development.

Merck has disclosed the structure and preclinical pharmacology of MK-6096, a second dual orexin receptor antagonist in clinical development.<sup>12,82</sup> MK-6096 is structurally distinct from suvorexant (Figure 16.4). This molecule is a potent receptor antagonist that is highly efficacious in promoting sleep in rats (3–30 mg/kg) and dogs (0.25–0.5 mg/kg). MK-6096 was reported to have entered phase II clinical studies in 2009.

### 16.3.3 OX<sub>2</sub>R-selective Antagonism

While dual orexin receptor antagonists have been shown to promote sleep in multiple preclinical species,<sup>11,12,73,76</sup> reagents with at least partial selectivity toward OX<sub>2</sub>R have been used to begin to evaluate the relative role of each receptor subtype in sleep architecture. An OX<sub>2</sub>R selective antagonist, JNJ-10397049 (Figure 16.5), along with an OX<sub>1</sub>R antagonist, SB-408124, has been used to evaluate the roles of these receptors in controlling arousal and sleep architecture. Each selective antagonist and Almorexant were dosed independently at 30 mg/kg 2 hours into the rat inactive period, resulting in sleep-promoting effects of Almorexant and OX<sub>2</sub>R selective antagonist, but no sleep effects of OX<sub>1</sub>R antagonism alone.<sup>35</sup> All compounds are brain penetrant, with JNJ-10397049 producing ~80% cortical OX<sub>2</sub>R occupancy for more than 6 hours, an OX<sub>2</sub>R occupancy matched by Almorexant at this dose. The sleep-promoting effects of JNJ-10397049-mediated OX<sub>2</sub>R antagonism included lengthening sleep bouts while 30 mg/kg Almorexant sleep-promoting effects were due to higher REM and NREM bout numbers. Temperature effects were seen with the 30 mpk Almorexant administration and rat activity effects tracked the sleep effects. This important study also tracked circulating levels of several



JNJ-10397049

**Figure 16.5** OX<sub>2</sub>R selective antagonist used for preclinical studies.

monamines to find histamine levels in LH decreased with Almorexant and OX<sub>2</sub>R-selective antagonist but not in response to SB-408124-mediated OX<sub>1</sub>R antagonism. SB-408124 did increase dopamine in prefrontal cortex as did Almorexant, but no cortical dopamine effects were seen following administration of the OX<sub>2</sub>R selective antagonist. Importantly, the sleep-promoting effects typically seen with OX<sub>2</sub>R-selective antagonism were attenuated when both the OX<sub>2</sub>R and OX<sub>1</sub>R selective antagonists were dosed together without altering the plasma level of either compound dosed alone. Together these results suggest OX<sub>2</sub>R antagonism is as effective as dual antagonism for producing sleep effects, and that OX<sub>1</sub>R antagonism has the capacity to exert a counteracting effect on the sleep-promoting properties of OX<sub>2</sub>R antagonism, perhaps by increasing cortical dopamine. Almorexant produced higher OX<sub>2</sub>R occupancy at these doses, which may explain why the sleep effects of this DORA equal those of a selective OX<sub>2</sub>R antagonist.

## 16.4 Orexin Function beyond Sleep

### 16.4.1 Orexin Signalling and Reward Pathways

From their location in hypothalamus, orexinergic neurons project to a wide range of sites throughout the brain, with dense innervation of key areas associated with arousal, reward and addictive behaviours. In addition to structures regulating arousal such as the locus coeruleus, basal forebrain and tuberomammillary nucleus, neurons expressing the orexin receptors are localized to regions associated with reward and stress including the nucleus accumbens, ventral tegmental area, dorsal raphe and amygdala. Orexin neurons interact with cholinergic, histaminergic, noradrenergic, serotonergic and dopaminergic neurons, highlighting the role of orexin neuropeptides in integrating a variety of stimuli. Support is growing for the hypothesis that modulating reward pathways by inhibiting orexin signalling may provide an opportunity for treating drug addiction and relapse.<sup>83–85</sup> In preclinical models, genetic and pharmacological antagonism of the orexin system results in reduced propensity for addictive behaviours, particularly with cue associations and in reinstatement models.

Several early studies suggested a role for orexins in reward processing and drug abuse, and the activation of orexinergic neurons in the lateral hypothalamus has been associated with drug seeking in preclinical models. Cocaine, morphine and amphetamine increase the activity of mesolimbic projections and, through excessive activation and alteration of dopamine levels, contribute to their addictive properties.<sup>86,87</sup> Orexinergic neurons become activated by cues paired to drugs of abuse such as cocaine, and reinstatement of extinguished cocaine-seeking can be induced by administration of orexin peptide.<sup>88–91</sup> Intriguingly, Zhang *et al.* demonstrated that chronic cocaine administration in rats causes a long-lasting up-regulation of OX<sub>2</sub>R protein in the nucleus accumbens, which persisted up to 60 days after discontinuation of cocaine



treatment, suggesting a link with cocaine-induced behavioural plasticity.<sup>92</sup> Further, orexin receptor antagonists are effective at blocking addictive behaviours in preclinical assays, including place preference produced by morphine, amphetamine sensitization, nicotine reinstatement and ethanol seeking.<sup>93–99</sup> Orexinergic neurons in the lateral hypothalamus were shown to respond to chronic morphine administration and subsequent withdrawal,<sup>88</sup> and are activated in conditioned place preference studies using morphine and cocaine as shown by Fos immunoreactivity.<sup>90</sup> There is an emerging link between orexin signalling to the VTA and addiction. Administration of OX-A into the VTA induced robust morphine preference in animals in which it had previously been extinguished, supporting the hypothesis that signalling from orexin neurons in the lateral hypothalamus to the VTA is involved in drug relapse.<sup>90</sup> Central administration of OX-A also reinstated cocaine seeking in rats, and appears to involve signalling through noradrenergic and CRF systems.<sup>89</sup> Orexin A induces synaptic plasticity in dopaminergic neurons of VTA, and behavioural sensitization to cocaine was shown to depend on orexin signals from the lateral hypothalamus.<sup>100</sup> More recently, several groups have pointed to a role for orexin in mediating the responsiveness of VTA dopaminergic neurons to glutamate signals and thereby increasing drug-seeking behaviors.<sup>91,100</sup> These observations indicate that there is a significant interaction between orexin signalling in the LH and the VTA in the reinstatement of extinguished drug-seeking behaviours, and that antagonism may provide an opportunity for therapeutic intervention.

The relative contribution of OX<sub>1</sub>R and OX<sub>2</sub>R to modulating addictive behaviours remains an area of intense focus. The majority of *in vivo* pharmacology studies implicate OX<sub>1</sub>R as playing the major role in reward-seeking behaviours, particularly cue-induced reinstatement. However, observations in some experiments using high doses of the OX<sub>1</sub>R antagonist, SB-334867, may be confounded by interactions with OX<sub>2</sub>R or other off-target activities. In fact, a number of behavioural pharmacology studies with OX<sub>2</sub>R antagonists indicate that OX<sub>2</sub>R activity contributes to responses in models of alcohol and morphine addiction.<sup>101,102</sup> Additional studies with highly selective and potent antagonists, combined with robust behavioural and neurochemical assays, should help to uncover the individual roles of the receptor isoforms.

The anatomical distribution and functional properties of the orexin system along with demonstration of orexin receptor antagonist efficacy in a range of behavioural models indicate the potential for orexin receptor modulators as possible therapeutic interventions to treat a variety of addictive disorders. A balance between the sleep-promoting effects and reinstatement blockade of orexin receptor antagonists will need to be obtained to provide viable therapeutic potential.

### 16.4.2 Orexin and Mood

Orexinergic neurons and orexin receptor expressing neurons are activated in response to stress and subsequently stimulate stress-related systems including

norepinephrine, dopamine and corticotrophin releasing hormone, pointing to a role for orexin signalling in depression and post-traumatic stress.<sup>103</sup> Although orexin signalling is broadly implicated in multiple preclinical models of depression, the specific contributions of orexins in these models are not entirely clear. In studies using clomipramine to induce depression in rats, treatment altered REM sleep architecture and increased prepro-orexin transcripts in hypothalamus and frontal cortex,<sup>104</sup> and may be an underlying cause for observed depressive-like behaviours in their earlier clomipramine-induced depression studies.<sup>105</sup> In another rodent study, OX-A administration reduced immobility in the rat forced-swim test, with pre-treatment of the orexin receptor antagonist SB-334867 blocking these effects.<sup>106</sup> Recently, Lutter *et al.* showed that intact orexin signalling was necessary for the efficacy of caloric restriction in a mouse model of depression.<sup>107</sup> After caloric restriction, wild-type mice show less immobility in the forced-swim test compared to prepro-orexin knockout mice. Similarly in a social defeat model, caloric restriction is efficacious in wild-type mice but not in prepro-orexin knockout mice. Additionally, genetic or pharmacological blockade of OX<sub>1</sub>R activity significantly reduced behavioural despair in mice, with OX<sub>2</sub>R mice showing increased despair behavior.<sup>108</sup> Nollet and colleagues reported a specific increase of orexin neuron activation in the dorsomedial hypothalamus in response to unpredictable chronic mild stress, which was reversed with the SSRI antidepressant fluoxetine. The authors further demonstrated that chronic treatment with Almorexant reduced behavioural despair in a tail-suspension assay.<sup>109</sup> Taken together, these studies indicate a potential role for orexin signalling dysregulation in preclinical depression models and in clinical affective disorders, which awaits further clinical investigation.

### 16.4.3 Orexigenic Roles

The name orexin, derived from the Greek “orexis” meaning appetite or longing, was ascribed by Sakurai *et al.* in their initial report on the behavioural effects of administration of the neuropeptide in rodents. Animals administered synthetic versions of hypocretin-1 (OX-A) or hypocretin-2 (OX-B) peptides exhibited increased wakefulness along with increased feeding behaviour. Because the mRNA and peptide had been isolated from the lateral/dorsomedial hypothalamus, a known hunger/satiety centre, these data were interpreted to reflect an increase in appetite.<sup>110,111</sup> Although the wake-promoting effects of orexin peptides have been widely replicated, along with clear pharmacological demonstrations of wake inhibition with orexin receptor antagonists, the role of this neurotransmitter in feeding behaviours is less well established, but remains an area of focused interest. In addition to appetite control, the hypothalamus has long been recognized to play a role in coordinating metabolic, neuroendocrine, arousal and behavioural responses to environmental cues. Given the restricted hypothalamic localization of orexinergic neurons, the orexin system is well situated to govern the integration of motivational, metabolic, sleep/wake

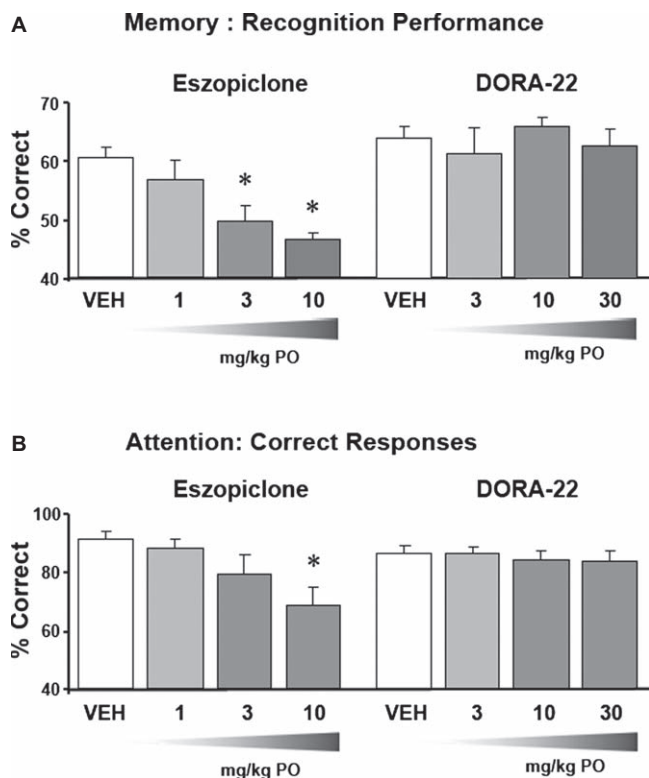
and autonomic processes required to maintain energy homeostasis. Several studies have reported increases in orexin-mediated feeding in species ranging from fish to mice to humans,<sup>112–114</sup> with interactions between NPY, nitric oxide, serotonin, acetylcholine and GABA signalling mechanisms implicated.<sup>115–119</sup> Interestingly, many of the patents covering orexin receptor antagonists indicate that these molecules may have potential therapeutic applications for obesity and metabolic disorders. Early efforts included the characterization of orexin receptor expression and screening for orexin receptor antagonists at Banyu laboratories with the aim of identifying new drugs for treating metabolic disorders.<sup>47</sup> Subsequently the focus of most pharmacological discovery efforts have shifted toward the impact of orexin receptor modulation for regulating sleep and wake using small-molecule antagonists.

## 16.5 OX<sub>R</sub> Antagonism Provides a Novel Therapeutic Alternative to Standard of Care for Insomnia

Orexin receptor antagonism represents a novel mechanism for the treatment of insomnia, offering potential advantages over current pharmacological therapies. The standard of care now includes non-benzodiazepine “z-drugs”, and zolpidem, zaleplon, zopiclone and eszopiclone, which interact with the benzodiazepine binding site of GABA<sub>A</sub> receptors, potentiating their activity leading to CNS depression.<sup>120–122</sup> GABA<sub>A</sub> receptors are widely expressed and function in a number of pathways including those associated with arousal, anxiety, psychomotor tone and cognition such that z-drug activity has the potential to have numerous other effects beyond sleep.<sup>123,124</sup> While these drugs are effective in attenuating sleep latency and promoting NREM sleep, they also suppress REM and slow wave components of normal sleep.<sup>125</sup> The most salient effect of Zolpidem in rats is the suppression of both the mean time spent in REM sleep and the number of REM sleep bouts, with comparatively slight reductions in active wake time.<sup>126</sup> This GABA<sub>A</sub> receptor-mediated somnolence is also associated with significant impairment of locomotor coordination not seen with dual OX<sub>R</sub> antagonists, Almorexant or SB-649868, even when the latter are administered up to 10-fold above their effective doses.<sup>73,127</sup> On the other hand, dual OX<sub>R</sub> antagonists such as suvorexant, MK-6096, DORA-22, Almorexant and SB-649868 promote somnolence that includes increases in both NREM and REM sleep in rodents and dogs.<sup>11,12,73,76</sup> The detailed evaluation of clinical effects of DORAs on REM and NREM will prove informative. Almorexant has been observed to increase both delta and theta EEG activity of human subjects, consistent with slow wave and REM sleep, while Zolpidem does not.<sup>76</sup>

Consistent with effects on multiple neurological pathways, eszopiclone administered to rhesus monkeys also impaired cognitive measures while DORA-22 induced no deficits, even when dosed at levels well in excess of the minimal effective dose for somnolence. In these studies eszopiclone significantly reduced the number of correct responses in both delayed match to

sample tasks and serial choice reaction time, together indicative of impaired memory and attention (Figure 16.6A, B, respectively). DORA-22, an analogue of MK-6096, however, showed no such detriments in the same measures. This lack of an effect of OXR antagonism on attention, learning and memory is consistent with Almorexant studies in rats, where no



**Figure 16.6** Eszopiclone, but not the Dual Orexin Receptor Antagonist, DORA-22, decreases recognition memory and attention performance in Rhesus monkeys. **A** Delayed Match to Sample (DMS) evaluation of eszopiclone or DORA-22 administered 80 and 60 min prior to behavioural testing, respectively. Values expressed as a mean percentage of completed trials on which a correct choice was made ( $\pm 1$  SEM;  $N = 6$ ). Random responding corresponds to a choice accuracy of 25%. **B** Serial Choice Reaction Time (SCRT) evaluation of eszopiclone or DORA-22 administered 170 and 140 min prior to behavioural testing, respectively. Data shown as a mean percentage of completed trials on which a correct choice was made ( $\pm 1$  SEM;  $N = 4$  (eszopiclone);  $N = 7$  (DORA-22)). Random responding corresponds to a choice accuracy of 10%. Significant differences from vehicle performance is indicated by \*,  $p < 0.05$  (Repeated Measures ANOVA, paired t-test).

Contributed by Spencer J. Tye, Department of *in vivo* Pharmacology, Merck Research Laboratories.

detriments were observed in spatial learning and memory tasks or avoidance retention.<sup>128</sup> Phase I studies with SB-649868, a novel DORA developed by GlaxoSmithKline, found the compound to be well tolerated with no evidence of next morning cognitive effects associated with improved total sleep time, decreased sleep latency and reduced WASO.<sup>74</sup> Effects of GABA<sub>A</sub> receptor modulation on learning and memory may be associated with rare amnesia-related side-effects including walking, eating and driving while asleep.<sup>124</sup> Whether these behaviours are secondary to REM suppression effects, involve amnesia-related anxiolytic mechanisms or the myriad pathways affected by GABA<sub>A</sub> receptor activity remains a point of debate and may vary depending upon GABA<sub>A</sub> receptor subtype selectivity.<sup>124,129</sup> Z-drugs also exhibit low to moderate risk of dependence, particularly in substance abusers and psychiatric patients,<sup>129,130</sup> while, at least preclinically, evidence indicates that OXR antagonists do not indicate evidence of tolerance or rebound.<sup>11,12,75</sup> Further work will determine if these favourable effects are due to direct modulation of orexin signalling or are a secondary consequence of the quality of sleep induced by OXR antagonists. Compared to the current standard of care, the prominent role of orexin in the control of arousal and vigilance state suggests that OXR antagonism represents a more selective mechanism of promoting somnolence resembling the natural reduction in orexin signalling that occurs during sleep.

## References

1. L. Lin, J. Faraco, R. Li, H. Kadotani, W. Rogers, X. Y. Lin, X. H. Qiu, P. J. de Jong, S. Nishino and E. Mignot, *Cell*, 1999, **98**, 365–376.
2. R. M. Chemelli, J. T. Willie, C. M. Sinton, J. K. Elmquist, T. Scammell, C. Lee, J. A. Richardson, S. C. Williams, Y. M. Xiong, Y. Kisanuki, T. E. Fitch, M. Nakazato, R. E. Hammer, C. B. Saper and M. Yanagisawa, *Cell*, 1999, **98**, 437–451.
3. C. Peyron, J. Faraco, W. Rogers, B. Ripley, S. Overeem, Y. Charnay, S. Nevsimalova, M. Aldrich, D. Reynolds, R. Albin, R. Li, M. Hungs, M. Pedrazzoli, M. Padigaru, M. Kucherlapati, J. Fan, R. Maki, G. J. Lammers, C. Bouras, R. Kucherlapati, S. Nishino and E. Mignot, *Nat. Med.*, 2000, **6**, 991–997.
4. S. Nishino, B. Ripley, S. Overeem, G. J. Lammers and E. Mignot, *Lancet*, 2000, **355**, 39–40.
5. R. A. Espana and T. E. Scammell, *Sleep*, 2011, **34**, 845–858.
6. S. Taheri, D. Sunter, C. Dakin, S. Moyes, L. Seal, J. Gardiner, M. Rossi, M. Ghatei and S. Bloom, *Neurosci. Lett.*, 2000, **279**, 109–112.
7. J. M. Zeitzer, C. L. Buckmaster, K. J. Parker, C. M. Hauck, D. M. Lyons and E. Mignot, *J. Neurosci.*, 2003, **23**, 3555–3560.
8. T. C. Thannickal, J. M. Siegel, R. Nienhuis and R. Y. Moore, *Brain Pathol.*, 2003, **13**, 340–351.

9. R. Fronczek, G. J. Lammers, R. Balesar, U. A. Unmehopa and D. F. Swaab, *J Clin. Endocrinol. Metab.*, 2005, **90**, 5466–5470.
10. T. Sakurai, *Nat. Rev. Neurosci.*, 2007, **8**, 171–181.
11. C. J. Winrow, A. L. Gotter, C. D. Cox, S. M. Doran, P. L. Tannenbaum, M. J. Breslin, S. L. Garson, S. V. Fox, C. M. Harrell, J. Stevens, D. R. Reiss, D. Cui, P. J. Coleman and J. J. Renger, *J. Neurogenet.*, 2011, **25**, 52–61.
12. C. J. Winrow, A. L. Gotter, C. D. Cox, S. M. Doran, P. L. Tannenbaum, M. J. Breslin, S. L. Garson, S. V. Fox, C. M. Harrell, J. Stevens, D. R. Reiss, D. Cui, P. J. Coleman and J. J. Renger, *Neuropharmacology*, 2011, in press.
13. C. T. Beuckmann and M. Yanagisawa, *J. Mol. Med.*, 2002, **80**, 329–342.
14. M. Hungs, J. Fan, L. Lin, X. Y. Lin, R. A. Maki and E. Mignot, *Genome Res.*, 2001, **11**, 531–539.
15. J. Hara, C. T. Beuckmann, T. Nambu, J. T. Willie, R. M. Chemelli, C. M. Sinton, F. Sugiyama, K. Yagami, K. Goto, M. Yanagisawa and T. Sakurai, *Neuron*, 2001, **30**, 345–354.
16. C. T. Beuckmann, C. M. Sinton, S. C. Williams, J. A. Richardson, R. E. Hammer, T. Sakurai and M. Yanagisawa, *J. Neurosci.*, 2004, **24**, 4469–4477.
17. S. Zhang, L. Lin, S. Kaur, S. Thankachan, C. Blanco-Centurion, M. Yanagisawa, E. Mignot and P. J. Shiromani, *Neuroscience*, 2007, **148**, 34–43.
18. N. Fujiki, T. Cheng, F. Yoshino and S. Nishino, *Exp. Neurol.*, 2009, **217**, 46–54.
19. T. E. Scammell, J. T. Willie, C. Guilleminault and J. M. Siegel, *Sleep*, 2009, **32**, 111–116.
20. T. Mochizuki, A. Crocker, S. McCormack, M. Yanagisawa, T. Sakurai and T. E. Scammell, *J. Neurosci.*, 2004, **24**, 6291–6300.
21. S. Kantor, T. Mochizuki, A. M. Janisiewicz, E. Clark, S. Nishino and T. E. Scammell, *Sleep*, 2009, **32**, 1127–1134.
22. C. G. Diniz Behn, E. B. Klerman, T. Mochizuki, S. C. Lin and T. E. Scammell, *Sleep*, 2010, **33**, 297–306.
23. T. Sakurai, M. Mieda, Y. Masashi, L. de Lecea and G. Sutcliffe, in *Hypocretins: Integrators of Physiological Functions*, Springer, New York, 2005, p. 27.
24. K. S. Williams and C. G. Behn, *J. Biol. Rhythms*, 2011, **26**, 171–181.
25. J. T. Willie, R. M. Chemelli, C. M. Sinton, H. Tokita, S. C. Williams, Y. Y. Kisanuki, J. N. Marcus, C. Lee, J. K. Elmquist, K. A. Kohlmeier, C. S. Leonard, J. A. Richardson, R. E. Hammer and M. Yanagisawa, *Neuron*, 2003, **38**, 715–730.
26. J. J. Hagan, R. A. Leslie, S. Patel, M. L. Evans, T. A. Wattam, S. Holmes, C. D. Benham, S. G. Taylor, C. Routledge, P. Hemmati, R. P. Munton, T. E. Ashmeade, A. S. Shah, J. P. Hatcher, P. D. Hatcher, D. N. C. Jones, M. I. Smith, D. C. Piper, A. J. Hunter, R. A. Porter and N. Upton, *Proc. Natl Acad. Sci. USA*, 1999, **96**, 10911–10916.
27. D. C. Piper, N. Upton, M. I. Smith and A. J. Hunter, *Eur. J. Neurosci.*, 2000, **12**, 726–730.



28. R. A. Espana, B. A. Baldo, A. E. Kelley and C. W. Berridge, *Neuroscience*, 2001, **106**, 699–715.
29. N. Fujiki, Y. Yoshida, B. Ripley, E. Mignot and S. Nishino, *Sleep*, 2003, **26**, 953–959.
30. D. Gerashchenko, M. D. Kohls, M. Greco, N. S. Waleh, R. Salin-Pascual, T. S. Kilduff, D. A. Lappi and P. J. Shiromani, *J. Neurosci.*, 2001, **21**, 7273–7283.
31. A. Yamanaka, S. Tabuchi, T. Tsunematsu, Y. Fukazawa and M. Tominaga, *J. Neurosci.*, 2010, **30**, 12642–12652.
32. Z. L. Huang, W. M. Qu, W. D. Li, T. Mochizuki, N. Eguchi, T. Watanabe, Y. Urade and O. Hayaishi, *Proc. Natl Acad. Sci. USA*, 2001, **99**, 1098–1098.
33. C. B. Saper, T. E. Scammell and J. Lu, *Nature*, 2005, **437**, 1257–1263.
34. A. Yamanaka, N. Tsujino, H. Funahashi, K. Honda, J. L. Guan, Q. P. Wang, M. Tominaga, K. Goto, S. Shioda and T. Sakurai, *Biochem. Biophys. Res. Commun.*, 2002, **290**, 1237–1245.
35. C. Dugovic, J. E. Shelton, L. E. Aluisio, I. C. Fraser, X. H. Jiang, S. W. Sutton, P. Bonaventure, S. J. Yun, X. R. Li, B. Lord, C. A. Dvorak, N. I. Carruthers and T. W. Lovenberg, *J. Pharmacol. Exp. Ther.*, 2009, **330**, 142–151.
36. P. Bourgin, S. Huitron-Resendiz, A. D. Spier, V. Fabre, B. Morte, J. R. Criado, J. G. Sutcliffe, S. J. Henriksen and L. de Lecea, *J. Neurosci.*, 2000, **20**, 7760–7765.
37. K. Ohno and T. Sakurai, *Front. Neuroendocrinol.*, 2008, **29**, 70–87.
38. M. I. Smith, D. C. Piper, M. S. Duxon and N. Upton, *Neurosci. Lett.*, 2003, **341**, 256–258.
39. M. Mieda, J. T. Willie, J. Hara, C. M. Sinton, T. Sakurai and M. Yanagisawa, *Proc. Natl Acad. Sci. USA*, 2004, **101**, 4649–4654.
40. A. R. Adamantidis, F. Zhang, A. M. Aravanis, K. Deisseroth and L. de Lecea, *Nature*, 2007, **450**, 420–424.
41. M. E. Carter, A. Adamantidis, H. Ohtsu, K. Deisseroth and L. de Lecea, *J. Neurosci.*, 2009, **29**, 10939–10949.
42. C. B. Saper, T. C. Chou and T. E. Scammell, *Trends Neurosci.*, 2001, **24**, 726–731.
43. S. Nishino, *Neuropeptides*, 2007, **41**, 117–133.
44. S. Nishino, M. Okuro, N. Kotorii, E. Anegawa, Y. Ishimaru, M. Matsumura and T. Kanbayashi, *Acta Physiologica*, 2010, **198**, 209–222.
45. M. Mieda, E. Hasegawa, Y. Y. Kisanuki, C. M. Sinton, M. Yanagisawa and T. Sakurai, *J. Neurosci.*, 2011, **31**, 6518–6526.
46. M. A. Akanmu and K. Honda, *Brain Res.*, 2005, **1048**, 138–145.
47. P. Trivedi, H. Yu, D. J. MacNeil, L. H. T. Van der Ploeg and X. M. Guan, *FEBS Lett.*, 1998, **438**, 71–75.
48. J. N. Marcus, C. J. Aschkenasi, C. E. Lee, R. M. Chemelli, C. B. Saper, M. Yanagisawa and J. K. Elmquist, *J. Comp. Neurol.*, 2001, **435**, 6–25.
49. T. Mochizuki, E. Arrigoni, J. N. Marcus, E. L. Clark, M. Yamamoto, M. Honer, E. Borroni, B. B. Lowell, J. K. Elmquist and T. E. Scammell, *Proc. Natl Acad. Sci. USA*, 2011, **108**, 4471–4476.



50. S. Yasuko, A. M. Atanda, M. Masato, Y. Kazuhiko and H. Kazuki, *Behav. Brain Res.*, 2010, **207**, 151–154.
51. H. S. Gompf and G. Aston-Jones, *Brain Res.*, 2008, **1224**, 43–52.
52. M. C. Xi, F. R. Morales and M. H. Chase, *Brain Res.*, 2001, **901**, 259–264.
53. K. Takahashi, Y. Koyama, Y. Kayama and M. Yamamoto, *Psychiatry Clin. Neurosci.*, 2002, **56**, 335–336.
54. L. Chen, J. T. McKenna, Y. Bolortuya, S. Winston, M. M. Thakkar, R. Basheer, R. E. Brown and R. W. McCarley, *Eur. J. Neurosci.*, 2010, **32**, 1528–1536.
55. M. C. Xi and M. H. Chase, *Sleep*, 2010, **33**, 1236–1243.
56. M. Kalogiannis, S. L. Grupke, P. E. Potter, J. G. Edwards, R. M. Chemelli, Y. Y. Kisanuki, M. Yanagisawa and C. S. Leonard, *Eur. J. Neurosci.*, 2011, **32**, 130–142.
57. D. Burdakov, *The Neuroscientist*, 2004, **10**, 286–291.
58. M. M. Thakkar, S. C. Engemann, K. M. Walsh and P. K. Sahota, *Neuroscience*, 2008, **153**, 875–880.
59. S. Satoh, H. Matsumura, T. Kanbayashi, Y. Yoshida, T. Urakami, T. Nakajima, N. Kimura, S. Nishino and H. Yoneda, *Behav. Brain Res.*, 2006, **170**, 277–286.
60. C. Broberger, L. de Lecea, J. G. Sutcliffe and T. Hokfelt, *J. Comp. Neurology*, 1998, **402**, 460–474.
61. L. Verret, R. Goutagny, P. Fort, L. Cagnon, D. Salvert, L. Leger, R. Boissard, P. Salin, C. Peyron and P. H. Luppi, *BMC Neurosci.*, 2003, **4**, 19.
62. M. Modirrousta, L. Mainville and B. E. Jones, *Eur. J. Neurosci.*, 2005, **21**, 2807–2816.
63. O. K. Hassani, P. Henny, M. G. Lee and B. E. Jones, *Eur. J. Neurosci.*, 2010, **32**, 448–457.
64. H. N. Brevig, C. J. Watson, R. Lydic and H. A. Baghdoyan, *Sleep*, 2010, **33**, 1285–1293.
65. T. Sakurai and M. Mieda, *Trends Pharmacol. Sci.*, 2011, **32**, 451–462.
66. T. Fenzl, C. P. Romanowski, C. Flachskamm, J. M. Deussing and M. Kimura, *Behav. Brain Res.*, 2011, **222**, 43–50.
67. T. Sakurai, M. Mieda and N. Tsujino, *Phylogenetic Aspects of Neuropeptides: from Invertebrates to Humans*, 2010, **1200**, 149–161.
68. C. R. Burgess, G. Tse, L. Gillis and J. H. Peever, *Sleep*, 2010, **33**, 1295–1304.
69. P. J. Coleman and J. J. Renger, *Expert Opin. Ther. Pat.*, 2010, **20**, 307–324.
70. C. Anaclet, R. Parmentier, K. Ouk, G. Guidon, C. Buda, J. P. Sastre, H. Akaoka, O. A. Sergeeva, M. Yanagisawa, H. Ohtsu, P. Franco, H. L. Haas and J. S. Lin, *J. Neurosci.*, 2009, **29**, 14423–14438.
71. A. C. Haynes, B. Jackson, H. Chapman, M. Tadayyon, A. Johns, R. A. Porter and J. R. Arch, *Regul. Pept.*, 2000, **96**, 45–51.
72. R. A. Porter, W. N. Chan, S. Coulton, A. Johns, M. S. Hadley, K. Widdowson, J. C. Jerman, S. J. Brough, M. Coldwell, D. Smart, F. Jewitt, P. Jeffrey and N. Austin, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 1907–1910.

73. R. Di Fabio, A. Pellacani, S. Faedo, A. Roth, L. Piccoli, P. Gerrard, R. A. Porter, C. N. Johnson, K. Thewlis, D. Donati, L. Stasi, S. Spada, G. Stemp, D. Nash, C. Branch, L. Kindon, M. Massagrande, A. Poffe, S. Braggio, E. Chiarparin, C. Marchioro, E. Ratti and M. Corsi, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5562–5567.
74. P. Bettica, G. Nucci, C. Pyke, L. Squassante, S. Zamuner, E. Ratti, R. Gomeni and R. Alexander, *J. Psychopharmacol.*, 2011 [Epub ahead of print].
75. C. Brisbare-Roch, M. Clozel and F. Jenck, *Sleep*, 2008, **31**, A38–A38.
76. C. Brisbare-Roch, J. Dingemanse, R. Koberstein, P. Hoefer, H. Aissaoui, S. Flores, C. Mueller, O. Nayler, J. van Gerven, S. L. de Haas, P. Hess, C. B. Qiu, S. Buchmann, M. Scherz, T. Weller, W. Fischli, M. Clozel and F. Jenck, *Nat. Med.*, 2007, **13**, 150–155.
77. K. Singletary, L. Simpson, C. Sagum and Y. Delville, *Sleep*, 2009, **32**, A32–A33.
78. P. Hoefer, S. de Haas, J. Winkler, R. C. Schoemaker, E. Chiossi, J. van Gerven and J. Dingemanse, *Clin. Pharmacol. Ther.*, 2010, **87**, 593–600.
79. P. J. Coleman, C. D. Cox and A. J. Roecker, *Curr. Top. Med. Chem.*, 2011, **11**, 696–725.
80. C. D. Cox, M. J. Breslin, D. B. Whitman, J. D. Schreier, G. B. McGaughey, M. J. Bogusky, A. J. Roecker, S. P. Mercer, R. A. Bednar, W. Lemaire, J. G. Bruno, D. R. Reiss, C. M. Harrell, K. L. Murphy, S. L. Garson, S. M. Doran, T. Prueksaritanont, W. B. Anderson, C. Tang, S. Roller, T. D. Cabalu, D. Cui, G. D. Hartman, S. D. Young, K. S. Koblan, C. J. Winrow, J. J. Renger and P. J. Coleman, *J Med. Chem.*, 2010, **53**, 5320–5332.
81. W. J. Herring, K. S. Budd, J. Hutzelmann, E. Snyder, D. Snively, K. Liu, C. Lines, D. Michelson and T. Roth, *Association of Professional Sleep Societies Annual Meeting*, San Antonio, TX, 2010, A0591.
82. P. J. Coleman, J. D. Schreier, C. D. Cox, M. J. Breslin, D. B. Whitman, G. B. McGaughey, R. A. Bednar, W. Lemaire, S. M. Doran, S. V. Fox, S. L. Garson, A. L. Gotter, M. Harrell, D. R. Reiss, T. D. Cabalu, D. Cui, T. Prueksaritanont, J. Stevens, P. L. Tannenbaum, R. G. Ball, J. Stellabott, G. D. Hartman, C. J. Winrow and J. J. Renger, *American Chemical Society Meeting, Division of Medicinal Chemistry*, 2011, MEDI 193.
83. G. C. Harris and G. Aston-Jones, *Trends Neurosci.*, 2006, **29**, 571–577.
84. G. Aston-Jones, R. J. Smith, G. C. Sartor, D. E. Moorman, L. Massi, P. Tahsili-Fahadan and K. A. Richardson, *Brain Res.*, 2010, **1314**, 74–90.
85. J. L. Thompson and S. L. Borgland, *Behav. Brain Res.*, 2011, **217**, 446–453.
86. L. J. Vanderschuren and P. W. Kalivas, *Psychopharmacology (Berl.)*, 2000, **151**, 99–120.
87. S. M. Anderson and R. C. Pierce, *Pharmacol. Ther.*, 2005, **106**, 389–403.
88. D. Georgescu, V. Zachariou, M. Barrot, M. Mieda, J. T. Willie, A. J. Eisch, M. Yanagisawa, E. J. Nestler and R. J. DiLeone, *J. Neurosci.*, 2003, **23**, 3106–3111.

89. B. Boutrel, P. J. Kenny, S. E. Specio, R. Martin-Fardon, A. Markou, G. F. Koob and L. de Lecea, *Proc. Natl Acad. Sci. USA*, 2005, **102**, 19168–19173.
90. G. C. Harris, M. Wimmer and G. Aston-Jones, *Nature*, 2005, **437**, 556–559.
91. G. Aston-Jones, R. J. Smith, D. E. Moorman and K. A. Richardson, *Neuropharmacology*, 2009, **56**(1), 112–121.
92. G. C. Zhang, L. M. Mao, X. Y. Liu and J. Q. Wang, *J. Neurochem.*, 2007, **103**, 400–407.
93. A. J. Lawrence, M. S. Cowen, H. J. Yang, F. Chen and B. Oldfield, *Br. J. Pharmacol.*, 2006, **148**, 752–759.
94. M. Narita, Y. Nagumo, S. Hashimoto, M. Narita, J. Khotib, M. Miyatake, T. Sakurai, M. Yanagisawa, T. Nakamachi, S. Shioda and T. Suzuki, *J. Neurosci.*, 2006, **26**, 398–405.
95. N. Cannella, D. Economidou, M. Kallupi, S. Stopponi, M. Heilig, M. Massi and R. Ciccocioppo, *Neuropsychopharmacology*, 2009, **34**, 2125–2134.
96. R. Sharf, M. Sarhan and R. J. DiLeone, *Brain Res.*, 2010, **1314**, 130–138.
97. C. J. Winrow, K. Q. Tanis, D. R. Reiss, A. M. Rigby, J. M. Uslaner, V. N. Uebele, S. M. Doran, S. V. Fox, S. L. Garson, A. L. Gotter, D. M. Levine, A. J. Roecker, P. J. Coleman, K. S. Koblan and J. J. Renger, *Neuropharmacology*, 2010, **58**, 185–194.
98. A. Plaza-Zabala, E. Martin-Garcia, L. de Lecea, R. Maldonado and F. Berrendero, *J. Neurosci.*, 2010, **30**, 2300–2310.
99. D. Quarta, E. Valerio, D. M. Hutcheson, G. Hedou and C. Heidbreder, *Neurochem. Int.*, 2010, **56**, 11–15.
100. S. L. Borgland, S. A. Taha, F. Sarti, H. L. Fields and A. Bonci, *Neuron*, 2006, **49**, 589–601.
101. Y. H. Li, H. Y. Wang, K. K. Qi, X. Y. Chen, S. Li, N. Sui and G. J. Kirouac, *Physiol. Behav.*, 2011, **102**, 42–50.
102. J. R. Shoblock, N. Welty, L. Aluisio, I. Fraser, S. T. Motley, K. Morton, J. Palmer, P. Bonaventure, N. I. Carruthers, T. W. Lovenberg, J. Boggs and R. Galici, *Psychopharmacology*, 2011, **215**, 191–203.
103. C. W. Berridge, R. A. Espana and N. M. Vittoz, *Brain Res.*, 2010, **1314**, 91–102.
104. P. Feng, Y. Hu, D. Li, D. Vurbic, H. Fan, S. Wang and K. P. Strohl, *J. Psychopharmacol.*, 2009, **23**, 559–566.
105. P. Feng, D. Vurbic, Z. Wu, Y. Hu and K. P. Strohl, *J. Psychopharmacol.*, 2008, **22**, 784–791.
106. N. Ito, T. Yabe, Y. Gamo, T. Nagai, T. Oikawa, H. Yamada and T. Hanawa, *Neuroscience*, 2008, **157**, 720–732.
107. M. Lutter, V. Krishnan, S. J. Russo, S. Jung, C. A. McClung and E. J. Nestler, *J. Neurosci.*, 2008, **28**, 3071–3075.
108. M. M. Scott, J. N. Marcus, A. Pettersen, S. G. Birnbaum, T. Mochizuki, T. E. Scammell, E. J. Nestler, J. K. Elmquist and M. Lutter, *Behav. Brain Res.*, 2011, **222**, 289–294.

109. M. Nollet, P. Gaillard, F. Minier, A. Tanti, C. Belzung and S. Leman, *Neuropharmacology*, 2011, **61**, 336–346.
110. T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R. M. Chemelli, H. Tanaka, S. C. Williams, J. A. Richardson, G. P. Kozlowski, S. Wilson, J. R. S. Arch, R. E. Buckingham, A. C. Haynes, S. A. Carr, R. S. Annan, D. E. McNulty, W. S. Liu, J. A. Terrett, N. A. Elshourbagy, D. J. Bergsma and M. Yanagisawa, *Cell*, 1998, **92**, 573–585.
111. L. de Lecea, T. S. Kilduff, C. Peyron, X. B. Gao, P. E. Foye, P. E. Danielson, C. Fukuhara, E. L. F. Battenberg, V. T. Gautvik, F. S. Bartlett, W. N. Frankel, A. N. van den Pol, F. E. Bloom, K. M. Gautvik and J. G. Sutcliffe, *Proc. Natl Acad. Sci. USA*, 1998, **95**, 322–327.
112. D. L. Choi, J. F. Davis, M. E. Fitzgerald and S. C. Benoit, *Neuroscience*, 2010, **167**, 11–20.
113. E. Yokobori, K. Kojima, M. Azuma, K. S. Kang, S. Maejima, M. Uchiyama and K. Matsuda, *Peptides*, 2011, **32**, 1357–1362.
114. K. Matsuda, K. S. Kang, A. Sakashita, S. Yahashi and H. Vaudry, *Ann. NY Acad. Sci.*, 2011, **1220**, 117–126.
115. M. Niimi, M. Sato and T. Taminato, *Endocrine*, 2001, **14**, 269–273.
116. G. Orlando, L. Brunetti, C. Di Nisio, B. Michelotto, L. Recinella, G. Ciabattini and M. Vacca, *Eur. J. Pharmacol.*, 2001, **430**, 269–272.
117. S. A. Farr, W. A. Banks, V. B. Kumar and J. E. Morley, *Peptides*, 2005, **26**, 759–765.
118. A. J. Thorpe, D. F. Doane, D. C. Sweet, J. L. Beverly and C. M. Kotz, *Brain Res.*, 2006, **1125**, 60–66.
119. D. Frederick-Duus, M. F. Guyton and J. Fadel, *Neuroscience*, 2007, **149**, 499–507.
120. E. Costa and A. Guidotti, *Annu. Rev. Pharmacol. Toxicol.*, 1979, **19**, 531–545.
121. S. S. Sullivan and C. Guilleminault, *Expert Opin. Emerging Drugs*, 2009, **14**, 411–422.
122. T. E. Scammell and C. J. Winrow, *Ann. Rev. Pharmacol. Toxicol.*, 2011, **51**, 243–266.
123. H. Ashton, *Drugs*, 1994, **48**, 25–40.
124. R. Hoque and A. L. Chesson, Jr, *J. Clin. Sleep Med.*, 2009, **5**, 471–476.
125. M. Lancel, *Sleep*, 1999, **22**, 33–42.
126. J. J. Renger, S. L. Dunn, S. L. Motzel, C. Johnson and K. S. Koblan, *Brain Res.*, 2004, **1010**, 45–54.
127. M. A. Steiner, H. Lecourt, D. S. Strasser, C. Brisbare-Roch and F. Jenck, *Neuropsychopharmacology*, 2011, **36**, 848–856.
128. H. Dietrich and F. Jenck, *Psychopharmacology (Berl.)*, 2010, **212**, 145–154.
129. K. R. Tan, U. Rudolph and C. Luscher, *Trends Neurosci.*, 2011, **34**, 188–197.
130. G. Zammit, *Drug Saf.*, 2009, **32**, 735–748.

## **Section 4**

# **Challenges and Future Directions in Drug Discovery for Psychiatric Disorders**



## CHAPTER 17

# *Crossing the Blood-brain Barrier – Methods for Evaluating CNS Exposure*

JAMES A. BAKER\* AND IAIN J. MARTIN

Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck  
Research Laboratories, 33 Avenue Louis Pasteur, Boston, MA 02115, USA

\*Email: james.baker5@merck.com

## 17.1 Introduction

### 17.1.1 Importance of Assessing CNS Penetration

A prerequisite for efficacy is the ability of a drug to reach the site of action. It has been commented that penetrating the CNS is a major hurdle in drug design, with > 98% of small molecules unable to cross the blood-brain barrier (BBB).<sup>1</sup> This widely quoted analysis was based on an assessment of likelihood of CNS activity (rather than CNS penetration) and so may underestimate the ease of small-molecule entry to the CNS.<sup>2</sup>

CNS penetration may also be important for compounds with peripheral targets but where CNS-mediated side-effects are anticipated. In such cases, peripheral restriction may be an objective. In either case, appropriate methods are required for assessing rate and extent of CNS penetration.

The physicochemical properties associated with good CNS penetration are reasonably well understood (*e.g.* moderate lipophilicity and low polar surface area<sup>3,4</sup>) and so medicinal chemistry for psychiatric indications tends to focus on



this chemical space. However, compounds that are expected, on the basis of their physicochemical properties, to have good brain penetration may be subject to other processes such as active efflux across the BBB. It is therefore prudent to assess CNS penetration early so that any barriers to central activity can be resolved during the optimization process.

The scope of this chapter is to focus on small-molecule drug design. For programs working with biologics, such as recombinant proteins, antibodies or small interfering RNA drugs, brain penetration is far more of a challenge. For these larger molecules, drug delivery approaches to the CNS are emerging.<sup>1,5</sup>

### 17.1.2 BBB Structure and Function

The BBB is composed of brain capillary endothelial cells, separating circulating blood from the brain interstitial fluid, which are characterized by having very limited paracellular transport due to the relative impenetrability of the intercellular tight junctions. Brain capillary endothelial cells have far higher transendothelial electrical resistance than peripheral capillaries ( $> 1,000$  versus  $< 20$  ohm/cm<sup>2</sup>) due to the tight junctions restricting movement of ions such as Na<sup>+</sup> and Cl<sup>-</sup>. This is thought to protect the brain from fluctuations in ionic composition that may occur, for example, due to exercise or a meal.<sup>6</sup> These cerebral microvessel endothelial cells are thought to adopt the BBB phenotype at least partly due to the influence of astrocytes, whose end-feet are in close proximity to the endothelial basal layer. The BBB tight junctions ensure that the predominant route of drug entry is by transcellular (passive) diffusion, emphasizing lipophilicity as a key determinant for CNS penetration.

The BBB is also characterized by its transporter expression pattern. There are several efflux and influx transporters expressed on the apical (blood) side of the endothelial cells. The International Transporter Consortium has recently agreed that good evidence exists for the transporters shown in Table 17.1 having functional activity at the human BBB.<sup>7</sup>

The uptake transporters have physiological importance in enabling the transport of essential nutrients into the brain, which would normally be excluded due to inappropriate physicochemical properties, for example, glucose, amino acids and nucleotides.<sup>8</sup> Both OATP1A2 and OATP2B1 are members of the solute carrier superfamily. Several drugs are also known to be taken into the brain by this uptake process, including fexofenadine and statins.<sup>7</sup> In contrast, the efflux transporters serve to eliminate both xenobiotics and endogenous compounds (e.g. cortisol by P-gp<sup>9</sup>) from the brain. All four of the efflux transporters in Table 17.1 are ATP-binding cassette proteins, using ATP to drive transport against a concentration gradient.

There are some species differences in transporter expression. For example, human P-gp is encoded by a single gene (*ABCB1*) while in rodents there are two homologues (*mdr1a* and *mdr1b*) with both rodent proteins sharing 90% amino acid homology with the human protein. Evidence suggests that rodent *mdr1a* is expressed at the BBB with *mdr1b* being expressed in brain parenchyma.<sup>10</sup>

**Table 17.1** Transporters found at the human BBB.<sup>7</sup>

<i>Transport direction</i>	<i>Transporter name</i>	<i>Aliases</i>	<i>Gene</i>
Uptake	Organic anion transporting polypeptide 1A2	OATP1A2 OATP-A	<i>SLCO1A2</i>
Uptake	Organic anion transporting polypeptide 2B1	OATP2B1 OATP-B	<i>SLCO2B1</i>
Efflux	P-glycoprotein	P-gp MDR1 ABCB1	<i>ABCB1</i>
Efflux	Breast cancer resistance protein	BCRP MXR	<i>ABCG2</i>
Efflux	Multi-drug resistance protein 4	MRP4 ABCC4	<i>ABCC4</i>
Efflux	Multi-drug resistance protein 5	MRP5 ABCC5	<i>ABCC5</i>

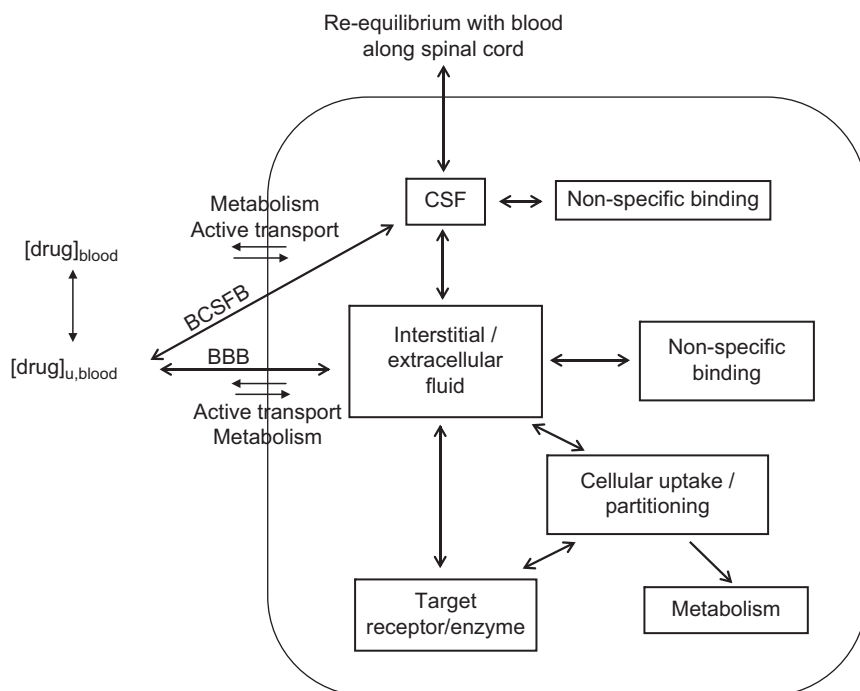
Pathological states can influence the composition and effectiveness of the BBB.<sup>6</sup> Conditions such as stroke, trauma, infection, inflammation, multiple sclerosis, neurodegenerative diseases, epilepsy and brain tumours can result in modulation of BBB function. This can be mediated through astrocyte secretion of growth factors and inflammatory mediators leading to changes in the expression of endothelial transporter and tight junction proteins.

### 17.1.3 Relationship between CNS Compartments

The brain has four main fluid compartments: (1) the blood, (2) the interstitial fluid (ISF; also known as ECF or extra-cellular fluid) bathing neurons and neuroglia, (3) cerebrospinal fluid (CSF) circulating around the ventricles and spinal cord and (4) intra-cellular fluid. The inter-relationships of the fluid compartments are illustrated in Figure 17.1.

The barrier separating the ISF from the CSF is a single layer of ependyma, a cell layer that does not have tight junctions. Thus, there is no major diffusional barrier between the ISF and the CSF.<sup>10,11</sup> The brain distribution of a number of clinically used drugs has been studied using microdialysis to access the drug concentrations in the ISF. These reports suggest that drug concentrations in CSF are a good approximation of those in brain ISF.<sup>12–16</sup> For some compounds, such as morphine-6-glucuronide, a very low CSF/ISF ratio has been attributed to slow equilibration into the CSF.<sup>17</sup> Several studies<sup>18</sup> have demonstrated the relationship between CSF concentrations and efficacy using preclinical models. For example, various infusion rates of phenobarbital resulted in loss of righting reflex in rats at the same CSF concentration despite differences in plasma and brain concentrations at that time.<sup>19</sup>

CNS penetration can be considered the sum of transport and permeability across the BBB and the blood-CSF barrier (BCSFB).<sup>4</sup> Although many publications focus on the BBB, it is now recognized that the BCSFB (comprised of



**Figure 17.1** Schematic illustrating the inter-relationship between drug in blood and CNS compartments. BBB is the blood-brain barrier and BCSFB is the blood-cerebrospinal fluid barrier.

the epithelial cells of the choroid plexus) can play a role in CNS penetration and cerebral detoxification.<sup>11</sup> The BCSFB is considered by some to have a surface area comparable to the BBB,<sup>11</sup> whereas others suggest a more limited size.<sup>17</sup> The BCSFB is considered more permeable than the BBB due to fenestrations, microvilli and leakier tight junctions. In addition, the BCSFB has its own complement of drug-metabolizing enzymes and transporters, the exact composition and orientation of which are the subject of debate.<sup>10</sup> The relative contribution of the BCSFB to brain penetration remains a controversial topic as, in addition to the surface area question, passive diffusion across the ependyma to the ISF may be counteracted by the bulk flow of the brain ISF to the CSF.<sup>20</sup>

As mentioned previously, the CSF is generally considered a good surrogate for the brain ISF concentration,<sup>10,17,21</sup> although caveats such as equilibration time have been mentioned and others will be discussed later (Section 17.3.2). In preclinical species (for example, rat, as described in detail later) the CSF can be sampled from the cisterna magna, *i.e.* proximal to the brain. However, in humans, CSF is usually sampled by direct aspiration of the lumbar CSF at a single time-point.<sup>10</sup> In addition CSF is not a well-stirred compartment and concentration gradients have been reported.<sup>10</sup> These factors mean that caution is needed when extrapolating from CSF to brain ISF levels in man.

## 17.2 Methods Commonly Used to Assess CNS Penetration

### 17.2.1 *In silico*

The use of *in silico* models to predict brain penetration has been extensively reviewed in the recent literature<sup>22–24</sup> (see also Chapter 18) and further review is beyond the scope of this chapter. Instead, we seek to provide some additional comment and perspective on this area.

In 2004 it was commented that existing *in silico* models of BBB permeability were built using log BB data and, whilst many of these models were reasonably accurate, the utility of such predictions was challenged.<sup>8,18</sup> The basis for this challenge was that log BB data, which are based on total drug concentrations, are not relevant to the drug available for pharmacological action (*i.e.* free drug concentrations). Furthermore, log BB data represent a composite of processes other than BBB permeability including plasma binding, tissue binding, efflux and clearance by interstitial flow.<sup>25</sup> Pardridge proposed that *in vivo* BBB Permeability Solute (PS) product should replace log BB as the standard for assessing BBB permeability.<sup>8</sup>

The utility of log BB as a descriptor of BBB permeability continues to be challenged.<sup>22,26,27</sup> Despite this, log BB is still the most frequently used experimental endpoint for the construction of *in silico* models.<sup>23,24,28</sup> A major reason for this must surely be the lack of publicly available *in vivo* BBB PS data, which in turn may be linked to the labour-intensive techniques needed to generate such data.

Drug penetration into the CNS is a multi-factorial process, therefore *in silico* models that adequately describe this will necessarily be complex. Attempts have been made to combine both rate (PS) and extent (log BB) of brain penetration into a single model<sup>29</sup> as well as link passive diffusion and P-gp-mediated efflux.<sup>26</sup> Nonetheless, the structural and physicochemical properties required for CNS penetration of small molecules are reasonably well understood<sup>22</sup> and may be readily and rapidly calculated. Therefore, the cost of generating additional *in vivo* data and *in silico* models may be too high in relation to the perceived additional benefit over existing permeability and transporter models.

### 17.2.2 *In vitro*

#### 17.2.2.1 *Permeability and Efflux*

*In vitro* tools are available to assess both permeability and transporter-mediated efflux, often within the same experimental system. This typically involves cell lines, selected for their ability to form confluent monolayers with apical and basolateral polarization, including Caco-2 (from human intestine), MDCK (from canine kidney) and LLC-PK1 (from porcine kidney). As indicated, none of these cell lines are brain endothelial in origin; however, they have been widely utilized as BBB surrogates, particularly for compounds that

undergo passive transcellular transport. Especially useful are cell lines that have been transfected with transporters, allowing the influence of permeability and efflux to be dissected from each other.

The assays generally involve the addition of drug to the donor well and measurement of the concentration of drug in the receiver well at the end of the incubation period. Typically, initial drug concentrations are kept low (*e.g.* 1–5  $\mu\text{M}$ ) to minimize the potential for saturation of transporter processes. The data are expressed as the apparent permeability ( $P_{\text{app}}$ , nm/s or  $\times 10^{-6}$  cm/s) in both the apical to basolateral (A to B; apical as donor side) and basolateral to apical (B to A; basolateral as donor side) directions. The efflux ratio is then calculated as the B to A/A to B  $P_{\text{app}}$  ratio. For cell lines that have native transporter expression (*e.g.* P-gp in Caco-2 cells), the A to B  $P_{\text{app}}$  of transporter substrates may be a composite of passive permeation and active efflux. To address this, studies may be performed with transporter inhibitors (*e.g.* verapamil for P-gp) or by employing high concentrations in the donor well (*e.g.* 100  $\mu\text{M}$ ) to saturate potential active transport. Another approach is to use cell lines that have less native transporter expression to obtain an efflux ratio, and then repeat the study in the same cell line specifically transfected with the transporter of interest. The efflux ratio with and without transporters can then be used to generate a “ratio of ratios”, giving a cleaner index of the role of that transporter. In such a dual-model approach, permeability data are generally quoted from the cell line lacking the transporter. The power of this approach is also derived from the fact that species-specific transporters can be transfected. This can be very useful when wishing to extrapolate from preclinical rodent *in vivo* data to the clinic.

Each *in vitro* system needs to be calibrated using compounds with known permeability and efflux, for which the following values are typically observed.

Low permeability	$P_{\text{app}} < 100 \text{ nm/s}$ ( $10 \times 10^{-6} \text{ cm/s}$ )
High permeability	$P_{\text{app}} > 200 \text{ nm/s}$ ( $20 \times 10^{-6} \text{ cm/s}$ )
Active transport	B to A/A to B efflux ratio $> 2.5$

*In vitro* co-culture models derived from more relevant cell types are also being developed; a rat primary cell model with brain epithelial and glial cells is now commercially available.<sup>30</sup> Promising advances have also been made recently in human cerebral microvascular endothelial cell lines.<sup>31</sup> Such tools may prove to be very informative but as yet are not used widely for screening purposes in drug discovery.

### 17.2.2.2 Plasma Protein Binding

Plasma protein binding (PPB) is routinely measured in drug discovery by equilibrium dialysis, ultrafiltration or ultracentrifugation. PPB data can be used in conjunction with plasma concentrations or AUC (area under curve) to derive free plasma exposures. This unbound plasma exposure can be related to free CNS levels, thus indicating whether or not equilibrium has been achieved.

The free drug hypothesis states that free drug drives pharmacological effect. Recent literature has emphasized that changes in PPB per se do not usually affect oral  $AUC_{unbound}$ .<sup>21,32,33</sup> Therefore, since increasing fraction unbound ( $f_u$ ) will not lead to higher unbound exposure in the CNS, it is suggested that the key optimization focus for CNS drugs should be increasing potency, minimizing efflux and decreasing intrinsic clearance.<sup>33</sup>

### 17.2.2.3 Binding to Brain Homogenate

The equilibrium dialysis methodology used for the PPB assay can also be applied to brain binding. Typically this is performed using homogenized brain tissue that has been diluted with buffer; the data are extrapolated to estimate the binding in the original (“undiluted”) tissue. The approach may be used with *ex vivo* samples following drug administration or, more often, brain homogenates from control animals are spiked with drug at a known concentration to give *in vitro* binding. Brain binding data are used to estimate the unbound concentration in brain, which in turn can be compared with unbound concentration in plasma. Analysis suggests that estimating free brain concentrations using this approach gives comparable data to CSF.<sup>34</sup>

It has recently been demonstrated that brain binding is species independent, based on an analysis of 47 diverse compounds in brain homogenates from 7 species and strains.<sup>35</sup> This information allows brain tissue binding measurements in rat to be used as an estimate for other species in drug discovery paradigms.

### 17.2.2.4 Binding to Brain Slices

It has recently been suggested that brain slice methods may offer improvement over the brain homogenate approach due to it maintaining the cellular structure and the associated pH gradient that influence distribution of ionized drugs within tissues.<sup>36</sup> These authors further suggest a  $pK_a$ -dependent correction be applied to brain homogenate data for ionized compounds. The brain slice method may still offer an advantage over “corrected homogenate” data as the slices have functional active brain cell uptake.

In the brain slice method, fresh brain slices (*e.g.* 300  $\mu\text{m}$  slices from drug-naïve rat brains) are incubated with drugs at very low concentrations (100 nM).<sup>37</sup> The drug concentration in buffer at the end of the incubation is assumed to equal the unbound concentration in the slice. This approach estimates the unbound volume of distribution in brain ( $V_{u, \text{brain}}$ ), a value that will increase with both non-specific binding and specific cellular uptake.

## 17.2.3 In vivo

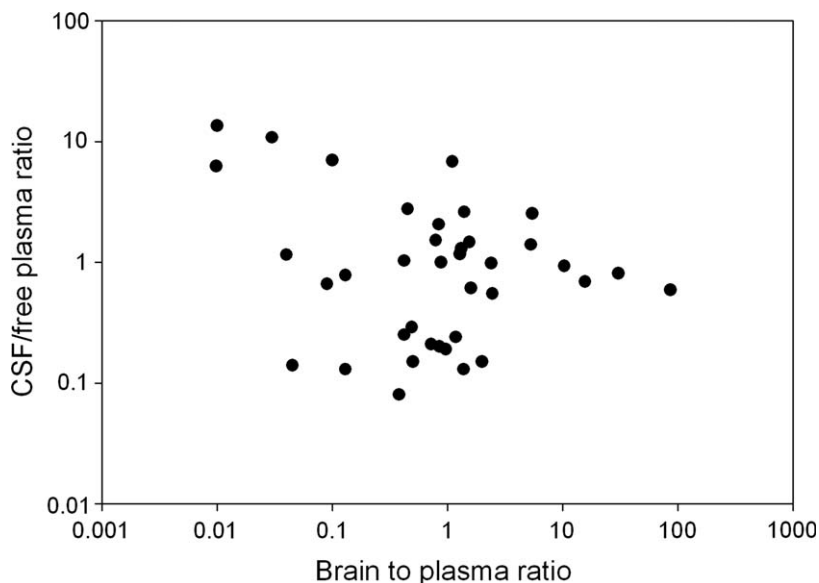
### 17.2.3.1 Total Brain to Total Plasma Ratio (*B/P ratio*)

Consistent with the free drug hypothesis, receptor occupancy studies have suggested that activity in the CNS is associated with free drug

concentrations.<sup>3,38,39</sup> In contrast, total brain concentrations, or B/P ratios, “reflects nothing but an inert partitioning process of drug into lipid material”<sup>3</sup> and is not indicative of drug available for pharmacological activity. Several authors have illustrated that focusing on increasing B/P ratios may mislead the optimization process by simply increasing non-specific brain binding.<sup>3,40</sup> Not only can this result in needless *in vivo* CNS efficacy studies<sup>3</sup> but it can also lead to compounds with high lipophilicity, and thus a reduced chance of suitable overall PK properties (*e.g.* high intrinsic clearance).

B/P data from the authors’ lab was shown to have no relationship with CSF/free plasma ratio (Figure 17.2). Additionally, CSF/free plasma ratios spanned two orders of magnitude compared to four for B/P ratio data. This indicates that B/P data are not useful for ranking compounds in terms of free drug availability in the CNS consistent with other reports.<sup>34,41</sup>

Total brain to total plasma ratio data may have value in the following scenarios: (1) in studies with knockout (KO) animals (see Section 17.2.3.2), (2) where the total brain levels are converted to free brain levels using binding data, (3) where a total brain to total plasma ratio of  $>0.05$  is used to show that some brain penetration is observed (*i.e.* brain levels are likely to be due to more than the brain vascular volume of blood<sup>41</sup>) and (4) to examine the temporal relationship of brain and plasma drug levels to identify delays in reaching equilibrium.



**Figure 17.2** B/P ratio as a predictor for CSF/plasma free ratio from studies in male Wistar rats.



### 17.2.3.2 Knockout (KO) Studies

KO mice lacking both *mdr1a* and *mdr1b* expression (*Mdr1a/1b*  $-/-$ ,  $-/-$ ) or *mdr1a* only (*Mdr1a*  $-/-$ ) are commercially available and can be used to compare total brain to plasma ratios in KO mice to those in their wild-type counterparts (e.g. FVB mice).<sup>41–43</sup> Since the brain tissue binding and PPB are assumed to be constant in both mouse genotypes, the comparison of the B/P ratios in KO *versus* wild-type is equivalent to a comparison of the unbound brain to unbound plasma ratios. Studies are generally performed with discrete groups at several time-points to allow AUC ratios from brain and plasma to be obtained and to study temporal profiles.

KO studies allow *in vivo* verification of the cause of low CNS penetration, perhaps following assessment in a serial CSF model. Such data give confidence in using *in vitro* models of P-gp efflux as a tool for selecting compounds likely to have improved CNS penetration. KO models for other transporters are available (e.g. BCRP  $-/-$ ). Interestingly, results from double KO mice studies (with *Mdr1a/b*  $-/-$ , BCRP  $-/-$ ) were initially interpreted as synergy between transporters at the BBB<sup>44</sup> although subsequently these effects have been considered simply additive.<sup>45,46</sup>

### 17.2.3.3 CSF Studies

In a drug-discovery environment, the rat is the usual CSF model although, occasionally, non-human primate models are also used. The conscious rat serial CSF model is described and the authors' preference for this model over terminal methods is discussed later.

**17.2.3.3.1 Surgical Preparation.** Anaesthetized rats are placed in a stereotaxic frame and the top of the head shaved and wiped with antiseptic solution. Antibiotics and appropriate analgesic agents (e.g. carprofen or vetgesic) are administered subcutaneously to minimize potential for post-operative discomfort. After the skull is exposed, holes are made in the left and right parietal bones and stainless steel anchor screws inserted, as shown in Figure 17.3(b).

A 2.3 mm trephine drill bit is then used to remove a piece of intra-parietal bone as shown in Figure 17.3(b), taking care not to damage the dura or cerebellum surface. The cannula with stylet (Figure 17.3(a)) is inserted in a near horizontal position to the occipital bone and then gently slid down the back of the skull to the cisterna magna (Figure 17.3(c)). The stylet can then be removed briefly to check CSF flow to confirm accurate placement.

The surface of the skull is dried and the base of the cannula and exposed skull covered in adhesive.

**17.2.3.3.2 Post-surgery.** CSF from the singly housed rats is sampled twice daily for the two days following surgery both to confirm CSF flow and to

**(a) Cannula and stylus details**

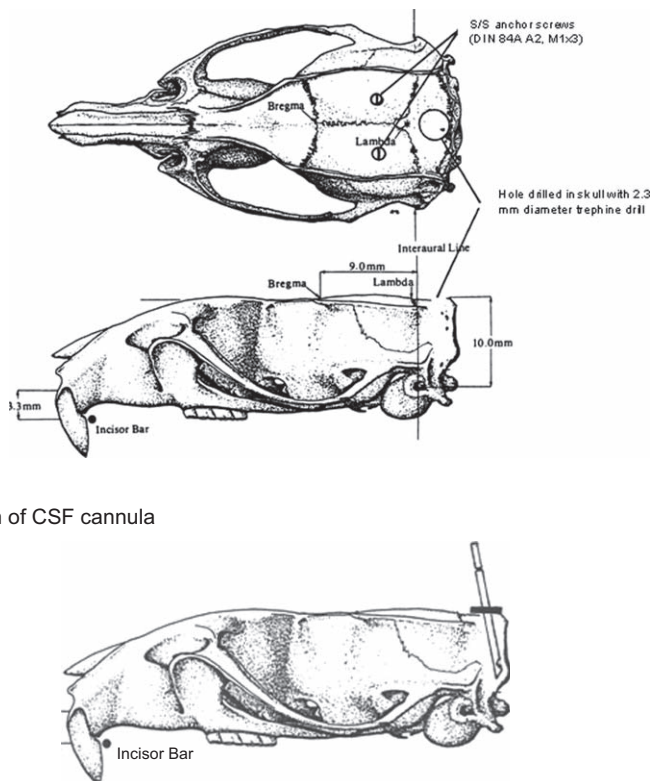
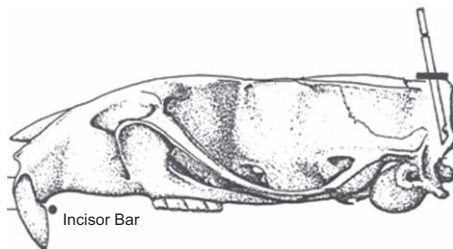
19G stainless steel guide cannula; 15 mm, ID 1.1 mm, OD 0.7 mm. Angled slot.

M1.6 flat DIN 125 washer (stainless steel) soldered onto cannula; 0.3 mm thick, ID 1.7 mm OD 4.0 mm.



19G stainless steel guide cannula; ID 1.1 mm, OD 0.7 mm. Crimped on 23G cannula

23G stainless steel guide cannula; OD 0.6 mm. Slightly bent. Solder to seal base.

**(b) Rat skull incision locations****(c) Final location of CSF cannula**

**Figure 17.3** Detail from the rat serial CSF model (diagrams (b) and (c) adapted from ref. 56 with permission).

check blood contamination. The study is typically performed on the third day after surgery.

**17.2.3.3.3 Serial CSF Study.** The total CSF volume in rat is  $\sim 250 \mu\text{l}$  and is replaced at a rate of approximately  $1\%/min$ .<sup>20</sup> To avoid a net depletion of total CSF volume,  $30\text{--}50 \mu\text{l}$  CSF samples are removed at a minimum interval of 15 minutes. A typical sampling scheme would be 0.5, 1, 2, 3, 4 and 6 h post-dose. CSF is collected into plain (unheparinized) glass capillary tubes.

To avoid non-specific binding to glass, CSF samples can be immediately diluted with an equal volume of acetonitrile. As well as sampling CSF, blood samples (*e.g.* from a tail vein) are taken at each time-point (usually for bioanalysis as plasma). Once the final CSF and blood samples are taken, terminal brain samples are also taken following deep anaesthesia with isoflurane. Blood or plasma and CSF samples are protein precipitated prior to sample analysis. Brains are homogenized with 3 volumes of ice-cold PBS prior to protein precipitation. Sample analysis is typically by LC-MS/MS with quantification against a calibration curve prepared in a suitable matrix (*e.g.* artificial CSF).

**17.2.3.3.4 CSF Study Data Analysis.** Plasma and CSF  $C_{\max}$  and  $AUC_{\text{last}}$  values are calculated by non-compartmental analysis; unbound plasma parameters are derived using *in vitro* plasma protein binding data. Study conclusions are based upon CSF/free plasma  $AUC_{\text{last}}$  ratios, where values of 0.3–3 are considered to reflect freely diffusible drug (*i.e.* unity  $\pm$  biological and experimental variability). Values of  $<0.3$  could indicate slow diffusion and/or active transport.

## 17.2.4 Microdialysis

Microdialysis offers the opportunity directly to measure the unbound drug levels in the brain (ISF), often with the simultaneous measurement of blood concentrations.<sup>47</sup> This powerful approach can reduce animal use and has also allowed the critical evaluation of the performance of other *in vivo* approaches (such as serial CSF). Microdialysis is a delicate technique with many experimental variables (*e.g.* probe construction, flow rate, tissue integrity following implantation) and also requires highly sensitive analytical methods due to the dilution of the sample with perfusion media.<sup>48</sup> An additional step for microdialysis compared with other approaches is the requirement for incorporation of recovery, which can vary for *in vitro* and *in vivo* measurements.<sup>48</sup>

Microdialysis is able to give very detailed information, such as drug distribution within the brain and quantitation of transport processes.<sup>47</sup> However, due to its complexities, the method has generally had limited use within drug discovery as a primary means of determining drug levels in the CNS. Nevertheless, pharmacodynamic studies employing brain microdialysis offer excellent opportunities for the simultaneous generation of pharmacokinetic data.

## 17.2.5 Receptor Occupancy

Drug concentration in the CNS can be inferred by the extent of target engagement, as determined by receptor occupancy (RO) studies, which measure competition with receptor-specific radioligands. In preclinical models, these studies are generally performed *ex vivo* on brain slices prepared following *in vivo* administration of the unlabelled compound of interest. In non-human

primates and clinically, RO is usually determined non-invasively by imaging methods such as positron emission tomography (PET)<sup>49</sup> with co-administered radio-labelled tracer ligand. RO studies provide an indirect measure of CNS penetration as the relationship between extent of ligand displacement depends on both the unbound concentration in the target tissue and binding affinity ( $K_i$ ). A limitation of the approach for assessing CNS penetration is that RO will not distinguish between the effect of parent drug and any active metabolites.

RO studies have provided valuable data to validate the relevance of previously described endpoints for assessing CNS penetration such as free brain concentration in preclinical models<sup>38,39</sup> and free plasma concentrations in humans.<sup>3</sup>

## 17.3 The Serial CSF Approach

### 17.3.1 Experiences of the Method

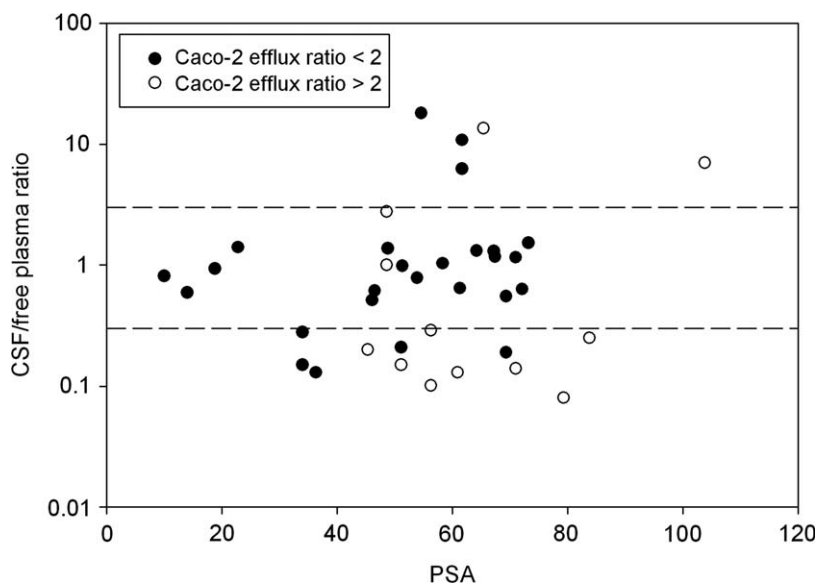
The method of choice for assessing CNS penetration at the authors' laboratory was the rat serial CSF model. The serial sampling approach was chosen in preference to terminal sampling for the following reasons:

- (a) Robust data were generally available from a serial sampling approach using only  $n = 4$  animals (compared to 20 rats for an equivalent terminal study).
- (b) CSF serial sampling provides time-courses from individual rats, thus improving data quality compared to reporting results using mean  $\pm$  standard deviation data at each time-point from discrete animals. The provision of serial sampling data may also be useful for determining pharmacokinetic/pharmacodynamic (PK/PD) relationships, particularly for compounds that are subject to active transport or a time lag in CNS penetration.
- (c) The use of terminal sampling raises the issue of which termination method to employ. A commonly reported method is asphyxiation with carbon dioxide. However, previous studies<sup>50</sup> have shown that this termination method can influence the amount of drug measured in plasma for basic compounds. Our recommendation is to take terminal blood and brain samples using isoflurane anaesthesia where drug concentration is being measured in any matrix (be it for CNS penetration, pharmacokinetic (PK) or PK/PD studies).

An essential requirement for experimental success was having highly skilled surgeons to implant the cisterna magna cannulae. This was important from the perspectives of both good post-surgical recovery rates and having quality CSF flow on the day of the study (volume and level of contamination). It was found that the degree of blood contamination was rat-strain dependent, with higher levels of blood contamination being observed in Sprague-Dawley compared with Wistar rats (data not shown).

Doses and administration routes were selected to ensure that concentrations of compound in the CSF were likely to be in a measurable range. Prior knowledge of rat PK and *in vitro* plasma protein binding were used to select the dose and route. Preference would be given to the target clinical dosing route; however, if rat oral bioavailability was low an alternative dosing route (typically subcutaneous) would be employed.

Data across several compound classes from the authors' laboratory are illustrated in Figure 17.4, where CSF/free plasma ratios are plotted against PSA. In this compound set (focused mainly on compounds with  $\text{PSA} < 80 \text{ \AA}^2$ ) 18 of 26 non-P-gp substrates showed CSF/free plasma ratios of 0.3–3. As discussed earlier, compounds that are able to diffuse freely into and out of the CNS and are not subject to active transport are expected to have CSF/free plasma ratios of  $\sim 1$ . Several publications have explored the relationship between *in vitro* efflux ratios and extent of brain penetration *in vivo*, with a good correlation being observed.<sup>51,52</sup> These data are largely derived from *mdr1a/b* knockout mouse studies. Data from in-house studies are summarized in Table 17.2, with rat CSF/free plasma ratios being compared to *in vitro* Caco-2 efflux ratios. Data that are well predicted by the *in vitro* model comprised 77% of compounds (*i.e.* high Caco-2 efflux, low CSF/free plasma ratio or no Caco-2 efflux, CSF/free plasma ratio  $\sim 1$  or higher). CNS efflux was over-predicted for 10% of compounds (Caco-2 efflux seen but CSF/free plasma  $> 0.3$ ). Under-prediction of CNS penetration, with relatively low CSF levels but no *in vitro* efflux, was observed in 13% of cases.



**Figure 17.4** Effect of PSA and Caco-2 efflux ratio on CSF/free plasma ratio in male Wistar rats (dashed lines indicate CSF/free plasma ratios of 0.3 and 3).

**Table 17.2** Relationship between Caco-2 efflux ratio (5  $\mu$ M in donor well) and CSF/free plasma AUC ratio data for 39 compounds from across a range of drug discovery projects.

		<i>CSF-free plasma ratio</i>	
		<i>&lt;0.3</i>	<i>&gt;0.3</i>
Caco-2 AtoB/BtoA efflux ratio	> 2	21% (8/39)	10% (4/39)
	< 2	13% (5/39)	56% (22/39)

Possible reasons for *in vitro* data not corresponding to the *in vivo* situation include (i) efflux assays being performed at concentrations  $>K_m$  leading to saturation of the transporter and (ii) efflux being mediated by transporters other than that found in the CNS. In the case of under-prediction, assays can be repeated at lower concentrations (as determined by bioanalytical sensitivity and/or comparability with *in vivo* CSF levels). If over-prediction of efflux is seen, the relevance of the Caco-2 (originally a colon carcinoma cell line) model could be assessed, for example by using P-gp specific inhibitors or by using transfected cell lines with minimal native transporter activity. Potential causes of misleading *in vivo* data are discussed in the next section.

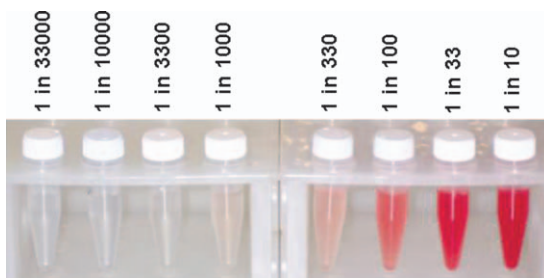
## 17.3.2 Factors Impacting CSF Data

### 17.3.2.1 High Protein Binding

CSF contains much lower protein levels than plasma ( $\sim 0.2$  vs.  $70$  mg/ml)<sup>4</sup> and is often considered to be “protein free”. Consequently, drug quantified in CSF is usually assumed to be unbound. However, compounds that have very high PPB may also have a significant degree of binding in CSF.<sup>4,17</sup> For example, if we assume the same binding constants in both matrices, a compound with 99.9% PPB would be 74% bound in CSF. Thus, where free drug equilibrium has been achieved, the measured CSF/free plasma ratio would be  $\sim 4$ . Consistent with this hypothesis, the three compounds in this data set with the highest PPB ( $>99.6\%$ ) had CSF/free plasma ratios  $>3$ . An additional factor when dealing with high PPB compounds is the ability to determine accurately the fraction unbound. For such compounds it may be advisable to use a plasma dilution protocol for assessing PPB.

### 17.3.2.2 Blood Contamination

One of the drawbacks of using CSF is the potential for blood contamination leading to higher measured drug concentrations. The impact of such contamination would be greater for compounds whose concentrations in blood are much higher than those in CSF (e.g. as a consequence of high PPB or erythrocyte partitioning). Figure 17.5 shows contamination on a visual scale (contamination of 1 in 330 or more can easily be seen by eye).



**Figure 17.5** Photograph of rat blood serially diluted in PBS. Contamination of higher than 1 in 330 can easily be discerned visually.

Methods for quantifying extent of CSF contamination were investigated in the authors' laboratory. It was found that absorbance at 405 nm (based on haemoglobin absorbance spectra) provided a robust, linear, quantitative method for quantifying blood contamination in both PBS and artificial CSF up to 1 in 1,000. Further studies indicated that it was possible to accurately back-calculate original drug concentrations in artificial CSF that had been spiked with blood containing differing concentrations of drug.

Consideration of the theoretical effect of blood contamination on CSF drug levels for varying degrees of PPB led us to propose the following rules-of-thumb:

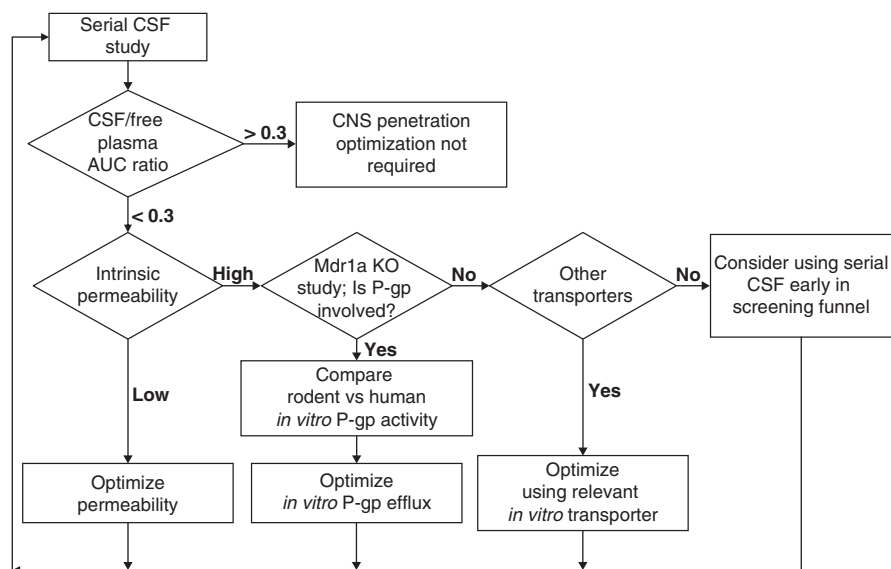
- Remove CSF data from the overall analysis where the samples are dark red (*i.e.* > 1 in 30 contamination).
- For compounds with PPB < 90%, blood contamination at < 1 in 30 is unlikely to be quantitatively significant.
- Where plasma protein binding is 90–97%, excluding CSF samples with visual coloration will maximize CSF data quality.
- Where plasma protein binding is > 97%, quantitation of degree of contamination may be useful as visual inspection may not identify samples that have potentially high blood levels. For such samples, a back-calculation approach may be suitable.
- If plasma protein binding is very high (> 99.9%), measured drug concentrations in CSF may be increased by both blood contamination and binding to CSF proteins. Caution should be therefore used when assessing the CNS penetration of such compounds.

## 17.4 Strategies for Optimizing CNS Penetration

A potential strategy for using various methods to optimize low CNS exposure is illustrated in Figure 17.6.

This reductionist approach aims to identify the cause of the low CNS exposure (*e.g.* permeability or active transport) and allows the project teams to select suitable screening strategies (preferably *in silico* or *in vitro*) to overcome





**Figure 17.6** A strategy for optimizing CNS penetration.

these challenges. Once improvement in the identified parameter is seen, compounds can be re-evaluated in the *in vivo* models (e.g. serial CSF or KO mouse model) to validate the screening strategy. Often this validation may be performed using compounds from the series that are relatively inactive at the biological target but that have the preferred DMPK properties.

Where the outlined approach does not identify *in silico* or *in vitro* tools for screening and optimization, the *in vivo* serial CSF model may be required earlier in the discovery screening flowchart. In such cases, approaches such as cassette dosing may be considered to increase throughput.

Compounds with low CNS penetration may require higher systemic exposure to achieve efficacy. This may have implications for dose size and systemic toxicity. Risperidone is a P-gp substrate that was shown to have a 10-fold higher B/P ratio in *mdr1a/1b* KO mice compared to wild-type mice,<sup>41</sup> yet is a successful psychiatric drug. However, the potential vulnerability of P-gp substrates to drug–drug interactions must also be considered.

## 17.5 Preclinical Species as Predictors for Human CNS Penetration

For most small molecules, the driving force for CNS penetration seems to be passive diffusion.<sup>49</sup> Thus membrane permeability is the main factor governing rate of drug uptake – a parameter not generally thought to be species dependent. Studies looking at the BBB in a wide range of species indicated a high level of structural homology across a diverse array of species.<sup>53</sup>

Active transport is more likely to be species-specific and, as noted earlier, rodents have both *mdr1a* and *mdr1b* in contrast to the single gene product in humans. Species differences in substrate specificity, tissue distribution and expression levels may be addressed by using species-specific *in vitro* models. A strong correlation between *in vitro* efflux by human MDR1 and mouse *mdr1a* using transfected MDCK cells has been demonstrated.<sup>54</sup> A high degree of spread was observed in the data set with, for example, a mouse *mdr1a* efflux ratio of 10 corresponding to human efflux ratios of 2–30. For this reason, cell lines transfected with human, mouse and rat *mdr1* homologues are routinely used in screening paradigms (see Section 17.2.2.1). It should also be noted that these *in vitro* models may not reflect any *in vivo* differences in expression levels. Interestingly, an analysis of CSF/free plasma ratios in humans compared with similar data from preclinical studies showed a good correlation, even for compounds that were substrates for transporters.<sup>17</sup>

The BBB is known to be affected by several pathologies that could result in differences in CNS exposure between patients, healthy volunteers and pre-clinical models.<sup>6</sup> In cases where CNS penetration is influenced by active transport, then drug–drug interactions may play a role in target organ exposure. This is exemplified by loperamide, an opioid drug used for the treatment of diarrhoea. When co-administered with quinidine, a P-gp inhibitor, the CNS availability of loperamide is increased resulting in centrally mediated side-effects (respiratory depression).<sup>55</sup>

## 17.6 Conclusions

Over recent years the emphasis of CNS drug optimization has shifted from parameters based on total drug concentrations to the appreciation of the need to optimize free drug concentrations –within both the systemic circulation and the CNS. In parallel, there has been increasing understanding that modulation of PPB will generally not yield increases in systemic or CNS exposure unless such changes are accompanied by improvements in key PK optimization parameters such as absorption and intrinsic clearance. Interestingly, although the relevance of total brain to total plasma ratios for optimization has been questioned, *in silico* models based on this parameter (or log BB) continue to be produced.

Literature opinion is divided as to the difficulty of achieving CNS penetration.<sup>1,3,49</sup> Experience from the authors' laboratory suggests that most small, drug-like molecules that are not effluxed readily penetrate the CNS and achieve CSF/free plasma ratios around unity. It should be highlighted that drugs access the CNS not only by crossing the BBB, but also through the less restrictive BCSFB. Perhaps the greater challenge in small-molecule drug discovery is the design of peripherally restricted yet orally bioavailable compounds.

For any psychiatry project, it is essential to understand the unbound CNS concentration in relation to the unbound plasma concentration. In this way,

any deviation from the free drug hypothesis can be identified and addressed. Such deviations typically result from active transport or low permeability.

## Acknowledgements

Thanks to all colleagues (project teams, DMPK and Bioanalysis) from Organon, Schering-Plough and MSD at Newhouse, Scotland, for their input to data generation and valued scientific discussions. Particular thanks to Rona Mason, Andrea Goodenough and Alan Byford for their considerable technical expertise.

## References

1. W. M. Pardridge, *Drug Discov. Today*, 2007, **12**, 54.
2. U. Fagerholm, *Drug. Discov. Today*, 2007, **12**, 1076.
3. H. van de Waterbeemd, D. A. Smith and B. C. Jones, *J. Comp.-Aided Molec. Design*, 2001, **15**, 273.
4. S. G. Jezequel, in *Progress in Drug Metabolism*, Taylor & Francis, London, 1992, **13**, p. 141.
5. K.K. Jain, in *Drug Delivery to the Central Nervous System*, ed. K. K. Jain, Springer, New York, 2010.
6. N. J. Abbott, L. Ronnback and E. Hansson, *Nat. Rev. Neurosci.*, 2006, **7**, 41.
7. K. M. Giacomini, S.-M. Huang, D. J. Tweedie, L. Z. Benet, K. L. R. Brouwer, X. Chu, A. Dahlin, R. Evers, V. Fischer, K. M. Hillgreen, K. A. Hoffmaster, T. Ishikawa, D. Keppler, R. B. Kim, C. A. Lee, M. Niemi, J. W. Polli, Y. Sugiyama, P. W. Swaan, J. A. Ware, S. H. Wright, S. Wah Yee, M. J. Zamek-Gliszczynski and L. Zhang, *Nat. Rev. Drug Discovery*, 2010, **9**, 215.
8. W. M. Pardridge, *Drug Discov. Today*, 2004, **9**, 392.
9. A. M. Karssen, O. C. Meijer, I. C. van der Sandt, P. J. Lucassen, E. C. de Lange, A. G. de Boer and E. R. de Kloet, *Endocrinology*, 2001, **142**, 2686.
10. J. H. Lin, *Curr. Drug Metab.*, 2008, **9**, 46.
11. W. Zheng, in *The Blood-Cerebrospinal Fluid Barrier*, CRC Press, 2005, p. xi.
12. Y. Deguchi, K. Inabe, K. Tomiyasu, A. Yamada and R. Kimura, *Pharm. Res.*, 1995, **13**, 647.
13. M. B. Hesselink, B. G. De Boer, D. D. Breimer and W. Danysz, *Pharm. Res.*, 1999, **16**, 637.
14. M. C. Walker, X. Tong, H. Perry, M. S. Alavijeh and P. N. Patsalos, *Br. J. Pharmacol.*, 2000, **130**, 242.
15. X. Wang and P. N. Patsalos, *Seizure*, 2003, **12**, 330.
16. X. Wang, N. Ratnaraj and P. N. Patsalos, *Seizure*, 2004, **13**, 574.
17. D. D. Shen, A. A. Artru and K. K. Adkinson, *Adv. Drug Deliv. Rev.*, 2004, **56**, 1825.

18. I. J. Martin, *Drug Discov. Today*, 2004, **9**, 161.
19. M. Danhof and G. Levy, *J. Pharmacol. Exp. Ther.*, 1984, **229**, 44.
20. M. Hammarlund-Udenaes, M. Frieden, S. Syvanen and A. Gupta, *Pharm. Res.*, 2008, **28**, 1737.
21. D. A. Smith, L. Di and E. H. Kerns, *Nat. Rev. Drug Discovery*, 2010, **9**, 929.
22. J. Mensch, J. Ovarzabal, C. Mackie and P. Augustijns, *J. Pharm. Sci.*, 2009, **98**, 4429.
23. S. Kortagere, D. Chekmarev, W. J. Welsh and S. Ekins, *Pharm. Res.*, 2008, **25**, 1836.
24. P. Garg, J. Verma and N. Roy, in *Drug Absorption Studies – In Situ, In Vitro, and In Silico*, ed. C. Ehrhardt and K.-J. Kim, Springer, New York, 2008.
25. U. Bickel, *J. A. Soc. Exp. Neurother.*, 2005, **2**, 15.
26. M. Adenot, in *Handbook of Neurochemistry and Molecular Neurobiology*, ed. A. Lajtha and M. E. A. Reith, Plenum, New York, 2007.
27. K. Lanevskij, P. Japertas, R. Didziapetris and A. Petrauskas, in *Drug Delivery to the Central Nervous System*, ed. K. K. Jain, Springer, New York, 2010.
28. L. Zhang, H. Zhu, T. I. Oprea, A. Golbraikh and A. Tropsha, *Pharm. Res.*, 2008, **25**, 1902.
29. K. Lanevskij, P. Japertas, R. Didziapetris and A. Petrauskas, *Chem. Bio-div.*, 2009, **6**, 2050.
30. N. Perriere, *Drug Metab. Reviews*, 2009, **41**(S3), 121.
31. S. Dauchy, F. Miller, P.-O. Couraud, R. J. Weaver, B. Weksler, I.-A. Romero, J.-M. Scherrmann, I. De Waziers and X. Decleves, *Biochem. Pharm.*, 2009, **77**, 897.
32. L. Z. Benet and B.-A. Hoener, *Clin. Pharmacol. Therapeut.*, 2002, **71**, 115.
33. X. Liu, C. Chen and C. E. C. A. Hop, *Curr. Topics Med. Chem.*, 2011, **11**, 450.
34. T. S. Maurer, D. B. DeBartolo, D. A. Tess and D. O. Scott, *Drug Metab. Disp.*, 2005, **33**, 175.
35. L. Di, J. P. Umland, G. Chang, Y. Huang, Z. Lin, D. O. Scott, M. D. Troutman and T. E. Liston, *Drug Metab. Disp.*, 2011, **39**, 1270.
36. M. Friden, F. Bergstrom, H. Wan, M. Rehngren, G. Ahlin, M. Hammarlund-Udenaes and U. Bredberg, *Drug Metab. Disp.*, 2011, **39**, 353.
37. M. Friden, F. Ducrozet, B. Middleton, M. Antonnson, U. Bredberg and M. Hammarlund-Udenaes, *Drug Metab. Disp.*, 2009, **37**, 1226.
38. X. Liu, O. Vilenski, J. Kwan, S. Apparsundaram and R. Weikert, *Drug Metab. Disp.*, 2009, **37**, 1548.
39. J. Watson, S. Wright, A. Lucas, K. L. Clarke, J. Viggers, S. Cheetham, P. Jeffrey and K. D. Read, *Drug Metab. Disp.*, 2009, **37**, 753.
40. P. Jeffrey and S. G. Summerfield, *Xenobiotica*, 2007, **37**, 1135.
41. A. Doran, R. S. Obach, B. J. Smith, N. A. Hosea, S. Becker, E. Callegari, C. Chen, X. Chen, E. Choo, J. Cianfrogna, L. M. Cox, J. P. Gibbs, M. A. Gibbs, H. Hatch, C. E. C. A. Hop, I. N. Kasman, J. LaPerle, J.-H. Liu,

- X. Liu, M. Logman, D. Maclin, F. M. Nedza, F. Nelson, E. Olson, S. Rahematpura, D. Raunig, S. Rogers, K. Schmidt, D. K. Spracklin, M. Szewc, M. Troutman, E. Tseng, M. Tu, J. W. Van Deusen, K. Venkatakrishnan, G. Walens, E. Q. Wang, D. Wong, A. S. Yasgar and C. Zhang, *Drug Metab. Disp.*, 2005, **33**, 165.
42. A. H. Schinkel, J. J. Smit, O. van Tellingen, J. H. Beijnen, E. Wagenarr, L. van Deemeter, C. A. A. M. Mol, M. A. van der Valk, E. C. Robanus-Maandag, H. P. J. te Riele, A. J. M. Berns and P. Borst, *Cell*, 1994, **77**, 491.
43. A. H. Schinkel, E. Wagenaar, C. A. Mol and L. van Deemter, *J. Clin. Invest.*, 1996, **97**, 2517.
44. J.W. Polli, K. L. Olson, J. P. Chism, L. John-Williams, R. L. Yeager, S. M. Woodard, V. Otto, S. Castellino and V. E. Demby, *Drug Metab. Disp.*, 2009, **37**, 439.
45. H. Kusuhara and Y. Sugiyama, *Drug Metab. Pharmacokinet.*, 2009, **24**, 37.
46. H. Kodaira, H. Kushara, I. Ushiki, E. Fuse and Y. Sugiyama, *J. Pharmacol. Exp. Ther.*, 2010, **333**, 788.
47. M. Hammarlund-Udenaes, *Adv. Drug Deliv. Rev.*, 2000, **45**, 283.
48. E. C. M. de Lange, A. G. de Boer and D. D. Breimer, *Adv. Drug Deliv. Rev.*, 2000, **45**, 125.
49. S. G. Summerfield, A. J. Lucas, R. A. Porter, P. Jeffrey, R. N. Gunn, K. R. Read, A. J. Stevens, A. C. Metcalf, M. C. Osuna, P. J. Kilford, J. Passchier and A. D. Ruffo, *Xenobiotica*, 2008, **38**, 1518.
50. D. A. Angus, J. A. Baker, R. Mason and I. J. Martin, *Drug Metab. Disp.*, 2008, **36**, 375.
51. C. Chen, E. Hanson, J. W. Watson and J. S. Lee, *Drug Metab. Disp.*, 2003, **31**, 312.
52. S. G. Summerfield and P. Jeffrey, *Expert Opin. Drug Discov.*, 2006, **1**, 595.
53. H. F. Cserr and M. Bundgaard, *Am. J. Physiol.*, 1984, **246**, R277.
54. B. Feng, J. B. Mills, R. E. Davidson, R. J. Mireles, J. S. Janiszewski, M. D. Troutman and S. M. de Morais, *Drug Metab. Disp.*, 2008, **36**, 268.
55. A. J. M. Sadeque, C. Wandel, H. He, S. Shah and A. J. J. Wood, *Clin. Pharm. Therap.*, 2000, **68**, 231.
56. G. Paxinos and C. Watson, in *The Rat Brain in Stereotaxic Coordinates*, Elsevier Academic Press, London, UK, 6th edn, 2007.

## CHAPTER 18

# *Medicinal Chemistry Challenges in CNS Drug Discovery*

MATILDA BINGHAM<sup>\*a</sup> AND ZORAN RANKOVIC<sup>\*b</sup>

<sup>a</sup> Newhouse, MSD, Motherwell, ML1 5SH, UK; <sup>b</sup> Eli Lilly and Company, 893 South Delaware Street, Indianapolis, IN 46285, USA

\*Email: m.bingham@redxpharma.com; rankovic\_zoran@lilly.com

## 18.1 Introduction

The modern medicinal chemist monitors an ever-increasing set of data variables representing the molecular properties deemed necessary for the optimal drug candidate. These include calculated physicochemical properties and *in vitro* and *in vivo* assay data used to predict efficacy, potency, safety and distribution metabolism and pharmacokinetic (DMPK) properties. Much of the data obtained and the strategies employed to address potential issues are common across therapeutic areas, for example addressing high blood clearance or cytochrome P450 inhibition. However, there is usually an additional set of data points specific to each therapeutic area and central nervous system (CNS) targets are no exception. This chapter will focus on the medicinal chemistry aspects of drug candidate optimization particular to the CNS therapeutic area, such as crossing the blood-brain barrier (BBB), as well as safety-related issues frequently challenging CNS programs such as hERG selectivity and phospholipidosis. Although there are also substantial pharmacological and clinical challenges in CNS research such as the poor predictivity of animal behavioural models and the availability of biomarkers, these areas are out of the scope of this chapter and are covered elsewhere in this book.

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

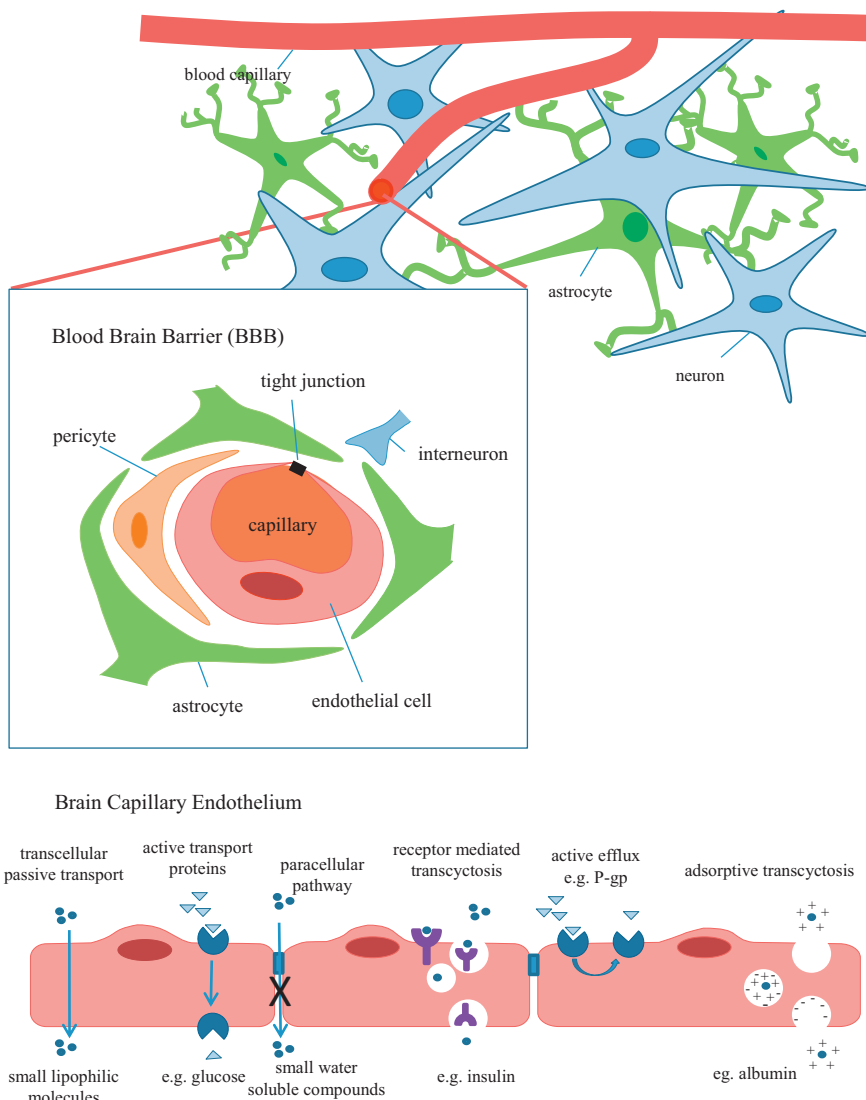
## 18.2 The Unique Challenge Posed by the Blood-brain Barrier

The human brain is a uniquely complex organ, which has evolved a sophisticated protection system to avoid injury from external insults and toxins. A significant challenge facing medicinal chemists working on CNS targets is the design of therapeutic agents that can overcome this protection system and enter the brain by crossing the blood-brain barrier and/or blood cerebrospinal fluid barrier (BBB/BCSFB) to achieve the drug concentrations required for efficacious target receptor occupancy in the brain region of interest. The blood-brain barrier (BBB) is found at the interface between the blood capillaries of the brain and brain tissue and is formed by three cell types: astrocytes, pericytes and endothelial cells, which line the blood vessels and form the brain capillary endothelium (Figure 18.1).<sup>1</sup> The BBB maintains brain homeostasis, regulating the access of nutrients, ions, hormones, solutes and proteins to the brain and protects the brain from harmful xenobiotics. As such it is very effective; whilst a range of essential circulating nutrients and endogenous molecules effectively penetrate the brain, often *via* selective active transport processes, more than 98% of small-molecule drugs and ~100% of large molecule drugs are excluded.<sup>2</sup>

The BCSFB is a similar barrier, albeit with a smaller surface area, which exists between the blood capillaries and the cerebrospinal fluid. The cerebrospinal fluid (CSF) is a clear liquid that fills the ventricles and canals of the brain and bathes the external surface providing buoyancy and mechanical protection to the brain inside the skull.<sup>3</sup> It is in exchange with the brain interstitial fluid (ISF) in brain tissue (Figure 18.2, *vide infra*) and provides a further means of drug access to brain tissue, in addition to its principle role in rinsing metabolic wastes from the CNS and regulating the chemical environment of the brain.

There are several processes by which molecules can enter the brain; however, research into targeting specific mechanisms for crossing the BBB is still in its infancy, and pharmaceutical CNS research is currently effectively limited to small-molecule therapeutics. The pharmaceutical industry has so far been slow to exploit transcytosis or transport proteins as mechanisms for drug delivery (Figure 18.1). An often-quoted example of exploiting an active transport mechanism is L-DOPA, which is used to treat Parkinson's disease;<sup>4</sup> however, this use of physiological protein machinery is an unusual approach in CNS therapeutics. Delivery systems based on temporal disruption of the BBB by nanoparticles, ultrasound or molecular Trojan horses are still in the research phase.<sup>5,6</sup> Furthermore, in contrast to the endothelial cells in capillaries elsewhere in the body, drug delivery to the brain by paracellular diffusion at the BBB/BCSFB is effectively precluded by the presence of tight junctions. Thus, the majority of CNS agents cross the BBB *via* the transcellular route (Figure 18.1). Many studies of the physicochemical properties required for passive transcellular transport across the BBB (*vide infra*) demonstrate that the criteria for small molecules to pass through the BBB endothelial cells are more





**Figure 18.1** Schematic representation of the blood-brain barrier (BBB), showing mechanisms by which small molecules and proteins can cross the BBB.

stringent than in other tissues. Moreover, the increased expression of active efflux transport systems such as the ATP binding cassette (ABC) transporter P-glycoprotein (P-gp, or ABCB1) adds an additional hurdle, and many drugs that effectively overcome or saturate efflux processes at the gut wall to obtain efficacious systemic concentrations still do not obtain appreciable concentrations in brain tissue. As a consequence the optimal design of CNS-penetrant medicines requires an understanding of the specific criteria for brain penetration.

### 18.2.1 Interpreting CNS Specific DMPK Methods to Facilitate Design of Centrally Acting Medicines

As a consequence of the effectiveness of the BBB, it is not sufficient to obtain classic DMPK data such as oral bioavailability and plasma fraction unbound levels when assessing CNS drug candidates since there is no certainty that these will mirror the time-course or exposure level seen in the brain. In order to gain an accurate picture of the free drug concentration in brain tissue, more sophisticated measures are required<sup>7–12</sup> (also see Chapter 17). Direct measurement of drug concentration in brain interstitial fluid is technically challenging and data sets providing this information are small. Moreover, receptor occupancy studies, which provide the most direct information on exposure at the site of action, are usually performed and reported for only the most advanced compounds within a drug-discovery programme. A compromise is found in the use of a range of surrogate techniques such as CSF sampling and brain  $F_u$  measurements, each in combination with information from plasma (iv/po) DMPK data and total brain concentration. The range of techniques available is discussed in more detail in the preceding chapter. Taken together, these data provide a more accurate picture of the brain free drug concentration  $C_{u,br}$  at the site of action. A list of the more commonly used measures of brain penetration along with their definitions is given in Table 18.1. These parameters are sometimes confused in the literature, particularly the fraction unbound in brain  $F_{u,br}$  and the free unbound drug concentration in brain  $C_{u,br}$ . To further confuse the issue, different pharmaceutical companies and research groups often choose to report data using differing terminology for each of the parameters. The commonly reported measures of brain penetration are grouped in Table 18.1, along with the most commonly reported acronyms, according to whether they represent a ratio ( $K_p$ ,  $K_{p,free}$ ,  $\text{LogBB}$ ,  $F_{u,br}$ ,  $F_{u,pl}$ ,  $\text{ER}$ ), a measure of rate of permeability ( $\text{PS}$ ,  $P_{app}$ ) or a measure of concentration in a particular compartment ( $C_{u,br}$ ,  $C_{u,pl}$ ,  $C_{CSF}$ ). Furthermore, Figure 18.2 is a simplified pictorial representation of drug distribution in the CNS and shows how the properties in Table 18.1 are interrelated, for example in the simple case where the free drug hypothesis holds true then at steady state the unbound drug concentration in brain ( $C_{u,br}$ ) and plasma ( $C_{u,pl}$ ) is the same.

Understanding how physicochemical properties affect each parameter and how the parameters influence each other enables the CNS medicinal chemist to design compounds with improved  $C_{u,br}$ . Dependent on which parameter is analyzed with respect to physicochemical properties, the conclusions will reflect the effect on the *rate of permeation* or the *extent of penetration* of the brain. To help put the values in context (Table 18.2), Reichel has reported a comparison between these parameters and the classical DMPK properties used for assessing oral exposure.<sup>13</sup>

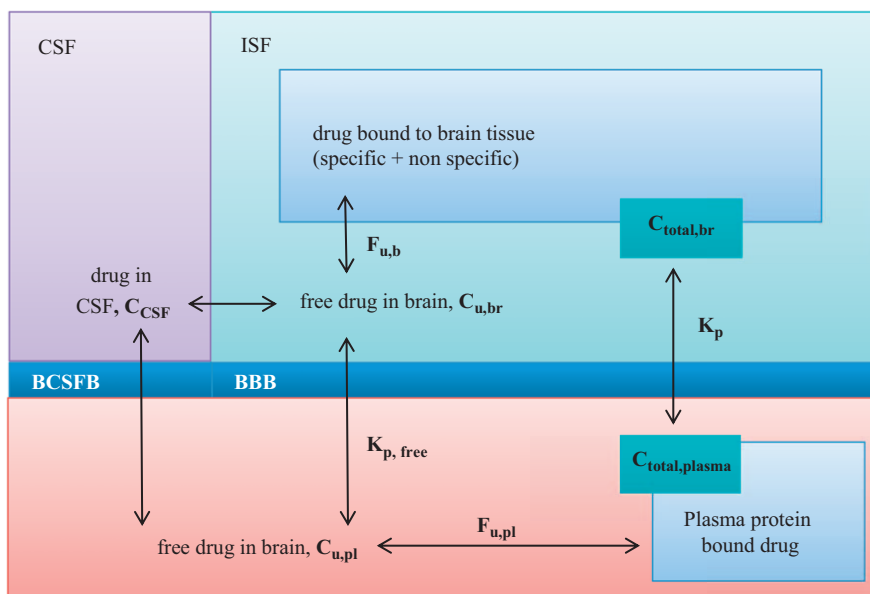
The free drug hypothesis states that the free drug concentration (as opposed to total drug concentration) at the site of action is responsible for the pharmacological activity and, in addition, it is assumed that at steady state, the free drug concentration is the same on both sides of any biomembrane.<sup>41</sup> As a result the  $C_{u,plasma}$  is often used to calculate the exposure required for efficacy based on the known  $K_i$  or  $\text{EC}_{50}$ . An assumption is thus made that the  $C_{u,plasma}$  reflects the unbound drug concentration in the tissue of interest since irrespective of

**Table 18.1** Definition of commonly reported measures of brain penetration.

Endpoint	Definition	Description	Units
<b>K<sub>p</sub></b>	$K_p = \frac{C_{brain}}{C_{plasma}}$	Brain to plasma distribution coefficient of total drug between brain and plasma.	No units (ratio)
B/P B:P Br:Bl		(Includes plasma protein bound and brain tissue bound drug, thus including non-specific binding to brain tissue.)	
<b>LogBB</b>	$LogBB = Log\left(\frac{C_{brain}}{C_{plasma}}\right)$ $LogBB = Log(K_p)$	Brain to plasma distribution coefficient of total drug between brain and plasma on a logarithmic scale.	No units (ratio)
<b>F<sub>u,br</sub></b> F <sub>u,brain</sub> ,	$F_{u,br} = C_{u,br}/C_{brain}$	Fraction unbound or Free drug fraction in brain tissue.	No units (ratio)
<b>F<sub>u,pl</sub></b> F <sub>u,plasma</sub> ,	$F_{u,pl} = C_{u,pl}/C_{plasma}$	Fraction unbound or Free drug fraction in plasma.	No units (ratio)
<b>K<sub>p,free</sub></b> (CSF/plasma)	$K_{p,free} = \frac{C_{u,brain}}{C_{u,plasma}}$	Brain to plasma distribution coefficient of unbound drug between brain and plasma. (Surrogate measure: CSF to plasma distribution coefficient.)	No units (ratio)
<b>ER</b>	$ERP_{app}(B \text{ to } A)/P_{app}(A \text{ to } B)$	Efflux ratio.	No units (ratio)
<b>PS</b>		Permeability surface area product.	μl/min/g brain
<b>P<sub>app</sub></b>		Permeability coefficient.	nm/s
<b>C<sub>u,br</sub></b> C <sub>free,br</sub>		Concentration of unbound drug in brain.	ng/g, nM
<b>C<sub>u,pl</sub></b> C <sub>free,pl</sub>		Concentration of unbound drug in plasma.	ng/g, nM
<b>C<sub>CSF</sub></b>		Concentration of drug in cerebrospinal fluid (CSF).	ng/g, nM

differential  $f_{u,plasma}$  and  $f_{u,tissue}$ , at steady state  $C_{u,plasma} = C_{u,tissue}$ . One of the challenges particular to CNS drug discovery is that often the BBB effectively limits access to the brain to the extent that this steady state assumption does not apply. Common situations where this can occur include:

- when a compound has low rate of passive permeability across the BBB/BCSFB;
- when the compound is actively pumped *out of* the brain by efflux transporters (for example P-gp);
- when the compound is actively transported *into* the brain by uptake transporters.



**Figure 18.2** A compartment model of drug transfer between plasma, brain and cerebrospinal fluid. (Definitions of acronyms and terms used are provided in Table 18.1.)

**Table 18.2** Reichel's conceptual analogy of "classic" PK and CNS PK.

	<i>"Classic" PK</i>	<i>CNS PK</i>
Rate	"Elimination" clearance $CL = \frac{Dose}{AUC_{plasma}}$	"Uptake" clearance $K_{in} = \frac{A_{brain}}{AUC_{plasma}}$
Extent	Extent of oral bioavailability $F = \frac{AUC_{po}}{AUC_{iv}}$	Extent of brain uptake $K_p = \frac{AUC_{brain}}{AUC_{plasma}}$
Distribution	Concept of total vs. unbound concentrations $V_u, f_{u,plasma}, C_{u,plasma}$	$V_{u,brain}, f_{u,brain}, C_{u,brain}$
Half-life	Half-life of elimination $T_{1/2,el} = \frac{\ln 2 \times V_{ss}}{CL}$	Half-life to equilibrium $T_{1/2,equ} = \frac{\ln 2 \times V_{u,brain}}{PS \times f_{u,brain}}$

$A_{brain}$  Amount of compound in brain corrected for intravascular content ( $\mu\text{g/g}$  brain).

In these cases there is disconnect between the compound's free drug concentration in plasma and brain (*i.e.*  $C_{u,plasma} \neq C_{u,brain}$ ). Ideally, a successful small-molecule CNS drug candidate will comply with the free drug hypothesis ( $C_{u,br} = C_{u,pl}$ , *i.e.*  $K_{p,free} = 1$ ).<sup>11</sup> It will have good BBB permeability ( $P_{app} > 150\text{--}200\text{ nm/s}$ ) and will not be subject to efflux ( $ER < 3$ ).<sup>14</sup> Therefore,

guidelines that provide direction on how to address the three issues highlighted above will facilitate identification of the optimal CNS drug candidate. It is perhaps instructive to comment here on the requirements for the whole brain-to-plasma ratio  $K_p$  (also called B/P, B:P, Br:Bl) since this is the most commonly reported parameter of brain penetration in the medicinal chemistry literature due to the relative ease of measurement. In cases where the starting compound for optimization has  $K_p \ll 1$  due to active efflux, by P-gp for example, then  $K_p$  can be a useful addition to the flow chart used as a tier I assay along with the measured efflux ratio (ER) from Caco-2 or MDR1-MDCK assays and whole blood clearance  $Cl_b$  data to prioritize compounds for further study, since changes in  $K_p$  may also enhance  $K_{p,free}$ . To confirm an effect on  $C_{u,br}$  this would need to be followed up with further experiments, such as a serial CSF study for example. However, in circumstances where no efflux transporter is involved then changes to  $K_p$  will only reflect differences in non-specific binding between brain and plasma and hence increases to the ratio  $K_p$  will not necessarily correlate with increased  $C_{u,br}$  (*vide infra*, Table 18.4).

There are several predictive models for passive permeability based on physicochemical properties, thus designing out poor permeability is the easiest of the three issues to tackle (*vide infra*). The *in vitro* models of BBB permeability (PAMPA, Caco2, MDR1-MDCK) are the same as those used to estimate permeability of the gut wall for evaluation of oral absorption. However, for CNS activity the implications of efflux and low permeability are more profound because the concentration of drug compound in brain blood capillaries is likely to be lower than in the gut, and thus saturation of efflux processes is unlikely to occur. As a result, although the influence of physicochemical properties shows similar trends for oral absorption and CNS permeability, the physicochemical property requirements for BBB permeability are usually more stringent. The Polli research group has shown in a study of 48 marketed CNS drugs and 45 marketed non-CNS drugs that, whilst 96% of CNS drugs demonstrate passive permeability  $P_{app} > 150$  nm/s in an MDR1-MDCKII assay only 76% of non-CNS drugs meet this criterion.<sup>14</sup> Thus the guideline of  $P_{app} > 150$ –200 nm/s was suggested for a compound with CNS exposure. In contrast Veber has suggested the widely quoted lower cut-off of Caco2 A to B  $P_{app} > 100$  nm/s as a guideline for obtaining good oral bioavailability.<sup>15</sup>

Due to the high expression levels of active efflux transporters at the BBB, efflux is also a common issue for compounds targeting the CNS. Given the number of transporters expressed in the endothelial cells of the blood-brain barrier and the heterogeneity of active sites even within individual transporters such as P-gp,<sup>16</sup> it is much harder to predict whether a molecule will be actively effluxed out of the BBB. Despite this caveat there is an increasing number of mnemonic models and emerging physicochemical rules that attempt to predict whether a molecule will be liable to active efflux. In general these studies have focused on P-gp as the main efflux transporter; however, there are a number of other transporters that are known to play an important role at the BBB (see Chapter 17).

Active transport in the form of active influx may have a positive impact on the brain exposure, particularly of compounds with poor inherent passive permeability. In these cases the problem is in understanding how the transport mechanism is related to molecular structure to the level that it can inform the design process. Once a clinical candidate is identified the suitable selection of DMPK experiments will enable pharmacokinetic/pharmacodynamic (PK/PD) studies, although concerns are likely to be raised with respect to inter-patient variability as potential transporter expression level differences may lead to toxicological outcomes or lack of efficacy. For these reasons the preferred optimization strategy for overcoming poor permeability of small-molecule drugs remains addressing inherent passive permeability rather than exploiting active transport.

### 18.2.2 Physicochemical Properties for CNS Penetration

By analogy with the now widely accepted Lipinski guidelines for the design of successful oral drugs,<sup>17</sup> the physicochemical properties for brain penetration have been studied and several groups have defined the characteristics of successful CNS drug candidates, using a variety of approaches (Table 18.3).

Inevitably the choice of data set is hugely influential on the conclusions drawn from these analyses and there is much debate in the CNS medicinal chemistry community on the best methods for evaluating brain penetration. When analyzing physicochemical property trends a balance frequently needs to be struck between assay throughput and data set size and variety, and physiological relevance of the experimental data. Many studies of large data sets have analyzed the physicochemical properties of marketed CNS drugs; therefore, the conclusions reflect not only brain penetration but also the DMPK and toxicological requirements for a successful CNS drug candidate.<sup>20</sup> Alternatively, researchers have analyzed large data sets of surrogate *in vitro* (Caco-2, PAMPA, MDR1-MDCK) BBB permeation data or crude *in vivo* measures of brain penetration such as brain:plasma ratios ( $K_p$  or LogBB). The former approach provides information on the *rate of permeation* of the BBB whereas the latter approach gives information on the *extent of penetration*, in this case the total drug concentration in brain. Used in isolation, neither technique provides insight to the more relevant brain free drug concentration ( $C_{u,br}$ ), hence the associated physicochemical guidelines need to be interpreted with care.

Based on the studies in Table 18.3 it can be seen that several key physicochemical properties have been identified that influence the rate of brain permeability and extent of brain penetration including H-bonding potential, molecular weight, lipophilicity, polar surface area (PSA), ionization state and rotatable bond count. It is often the case that no single physicochemical property can be used to predict a given parameter such as  $P_{app}$  for example, but rather multi-variate models incorporating several descriptors are required. These multi-variate models are very useful when applied as a filter to check chemists' ideas for future synthesis, often *via* an online tool. However, they can

**Table 18.3** Summary of proposed physicochemical guidelines for optimizing CNS penetration.

<i>Author</i>	<i>Year</i>	<i>Data set</i>	<i>Conclusions/criteria</i>	<i>Ref.</i>
Van der Waterbeemd and Kansy	1992	20 compounds	Propose that LogP alkane/water and calculated molar volume are suitable predictors of brain uptake.	18
Gratton <i>et al.</i>	1997	18 chemically diverse compounds with LogPS data	LFER equation relating LogPS to solute excess molar refraction & solute volume. ↑ solute dipolarity/polarizability and hydrogen bond basicity leads to ↓ LogPS.	19
Van der Waterbeemd <i>et al.</i>	1998	125 CNS and non-CNS drugs	MW < 450; PSA < 90 Å <sup>2</sup> ; LogD between 1 and 4; principal axis length/width ratio < 5.	20
Polli <i>et al.</i>	2002	48 CNS and 45 non-CNS drugs	Physicochemical properties with significant differences between CNS and non-CNS set. CNS set had: ↑ cLogP (CNS mean 3.43); ↑ cLogD (CNS mean 2.08); ↓ HBD (CNS mean 0.67); ↓ PSA (CNS mean 40.5 Å <sup>2</sup> ) and were less flexible.	14
Norinder and Haeberlein	2002	Literature review	For a good chance of CNS penetration: O + N ≤ 5 cLogP – (O + N) > 0	21
Petrauskas <i>et al.</i>	2003	1,000 P-gp substrate specificity data points	Suggested physicochemical cut-offs to avoid P-gp efflux liability: MW < 400; N + O < 4; pK <sub>a</sub> < 8	22
Leeson and Davis	2004	329 launched oral drugs from the period 1983–2002	CNS drugs showed significantly different (mean/median) MW (310/307); polar properties (O + N (4.32/4); HBA (2.12/2); Rot. bond (4.7/4.5)) relative to other therapeutic area categories.	23
Pajouhesh and Lenz	2005	Literature review	Attributes of a successful CNS drug candidate: MW < 450; cLogP < 5; HBD < 3; HBA < 7; Rot. bonds < 8; H-bonds < 8; pK <sub>a</sub> 7.5–10.5; PSA < 60–70 Å <sup>2</sup> .	24
Hitchcock and Pennington	2006	Literature review	Suggested physicochemical property ranges for increasing the potential for BBB penetration: PSA < 90 Å <sup>2</sup> ; HBD < 3; cLogP 2–5; cLogD (pH 7.4) 2–5; MW < 500	25



**Table 18.3** (Continued)

<i>Author</i>	<i>Year</i>	<i>Data set</i>	<i>Conclusions/criteria</i>	<i>Ref.</i>
Gleeson	2008	3,059 rat CNS LogBB data points 1,975 P-gp efflux ratio data points 50,641 P <sub>app</sub> artificial membrane assay data points 986 brain tissue binding data points	↑ MW ↓ LogBB. ↑ cLogP ↑ LogBB. ↑ MW ↑ P-gp efflux ratio. cLogP has a weak non-linear effect on efflux ratio; the optimal cLogP is < 3 or > 5. ↑ MW ↓ permeability. Permeability of neutral molecules shows a non-linear dependence on cLogP. Basic, acid and zwitterionic molecules show ↑ permeability with ↑ cLogP. ↑ MW ↑ brain tissue binding. ↑ cLogP ↑ brain tissue binding.	26
Waring	2009	9,571 AstraZeneca Caco-2 measurements	Permeability rules to achieve > 50% chance of high permeability for a given MW. MW < 300 AZLogD > 0.5; MW 300–350 AZLogD > 1.1; MW 350–400 AZLogD > 1.7; MW 400–450 AZLogD > 3.1; MW 450–500 AZLogD > 3.4; MW > 500 AZLogD > 4.5	27
Wager	2010	119 marketed CNS drugs and 108 Pfizer CNS clinical candidates	Median values for CNS drugs: MW 305.3, cLogP 2.8, cLogD 1.7; MW 305.3; PSA 44.8 Å <sup>2</sup> , HBD = 1, pK <sub>a</sub> 8.4	28
Wager	2010	119 marketed CNS drugs and 108 Pfizer CNS clinical candidates	Multi-parameter optimization tool based on weighted physicochemical properties including cLogP, cLogD, PSA, MW, HBD, pK <sub>a</sub>	29

be difficult for a medicinal chemist to intuit when designing a molecule “in one’s head”. In the latter case it is useful to have an idea of how each of the individual physicochemical properties influences CNS penetration and rate of permeation.

The challenge posed to the medicinal chemist is thus to realize the delicate balance of physicochemical properties and SAR to address deficits in one parameter without negatively impacting another. For example, increasing lipophilicity can improve BBB permeability, but may negatively impact blood clearance and raise non-specific binding, thus leading to an overall reduction in C<sub>u,brain</sub>. To achieve a suitable balance it is useful to have an idea of how each of the individual physicochemical properties influences the extent of CNS penetration and rate of permeation. For the purposes of this review each physicochemical property will be treated in turn with examples from the medicinal chemistry literature. Where relevant, the interdependences and correlations between physicochemical properties will be highlighted.

### 18.2.2.1 Lipophilicity

The influence of lipophilicity on all aspects of medicinal chemistry from drug-receptor interactions to drug metabolism and distribution is widely appreciated. As a consequence, the surrogate measures cLogP and cLogD are commonly monitored parameters from the earliest stages of the discovery process. The recognition that lipophilicity is an important determinant in brain penetration predates modern computational approaches to QSAR. Lipophilicity has since been shown to affect both the rate and extent of brain penetration.

Early studies using the Hansch approach demonstrated that an increased octanol-water partition coefficient (LogP) was associated with increased CNS activity and increased LogBB.<sup>30</sup> Subsequent studies using a selection of marketed CNS and CNS-inactive drugs have confirmed these observations and extended them to include the more physiologically relevant lipophilicity indicator, the octanol-water distribution coefficient (LogD), with values in the range of cLogD 1–4 being proposed as optimal for brain penetration.<sup>20</sup> A recent paper by Gleeson reviewed 3,059 diverse molecules from GSK with CNS penetration (LogBB) data, and demonstrated that ionization state was also important in defining the dependence on lipophilicity; neutral, acidic and basic molecules showed a trend for increased LogBB with increasing cLogP, but zwitterionic molecules showed no such correlation.<sup>26</sup> Although the correlation between lipophilicity and LogBB is now well documented, the relevance of this latter value to the availability of the drug molecule at the site of action is now questioned.<sup>7–12</sup> Gleeson has shown that brain tissue binding is correlated with cLogP, thus the higher LogBB values observed for more lipophilic compounds likely reflect increased non-specific brain tissue binding and will thus not necessarily correlate with the therapeutic effect.<sup>26</sup> Liu *et al.* have recently proposed that high  $F_{u,br}$  should be a target for optimization when a short onset of action is required,<sup>10</sup> hence Gleeson's study showing the negative correlation between  $F_{u,br}$  and LogP supports lowering of LogP.

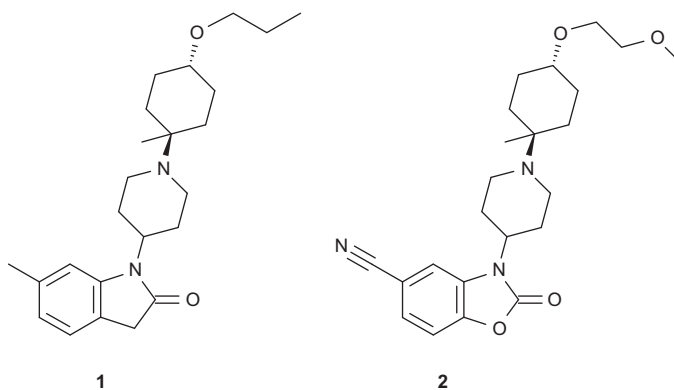
Lipophilicity has been shown to affect not only the *extent* of brain penetration in terms of LogBB and the extent of non-specific binding represented by  $F_{u,br}$ , but also the *rate* of permeation of the BBB. The relationship with permeability is non-linear<sup>26,31,32</sup> and the influence of lipophilicity is usually dependent on molecular volume, ionization state and H-bonding potential as represented by a descriptor such as PSA, H-bonding potential, LogD or MW. Some of these descriptors are correlated with each other, for example higher MW has been shown to correlate with increased PSA. As a result there are a number of simple models of CNS permeability that use an indicator of lipophilicity with just one other descriptor to predict rate of permeation. In an analysis of over 50,000 measurements in an artificial membrane (PAMPA) assay from GSK Gleeson showed that for acidic, basic and zwitterionic molecules increasing LogP increased passive permeability on average; however, for neutral molecules there was no such linear correlation.<sup>26</sup> Waring studied a data set of 9,571 compounds from AstraZeneca's Caco2 assay and concluded that LogD was a better descriptor to use than LogP.<sup>27</sup> He demonstrated that as

MW increased LogD needed to increase to maintain a 50% chance of high permeability so that for a compound of 300–350 MW the cLogD requirement was  $>1.1$ , but for a compound of 450–500 Da cLogD should be  $>3.4$ . Martin has also shown the utility of MW and LogD as predictors for permeability in a study of rat oral bioavailability; of 103 compounds with LogD  $<2.5$  and MW  $>500$ , 84% were classed as not permeable in Abbott's Caco2 assay.<sup>33</sup> In a slightly more complex model to set up, but a simple model to use and interpret, Egan and coworkers have described a “confidence ellipse” based on ALogP98 and PSA, which was used at Pharmacopeia to predict Caco2 permeability.<sup>34</sup> Again the selected  $P_{app}$  cut-offs focused on oral absorption but the principles will also apply to BBB permeation. Similar dependencies of the BBB permeability surface area product (PS or LogPS) on LogD and molecular weight have been described,<sup>8,35–37</sup> although the more recent models of LogPS values have provided computational models with more than two descriptors.<sup>38</sup>

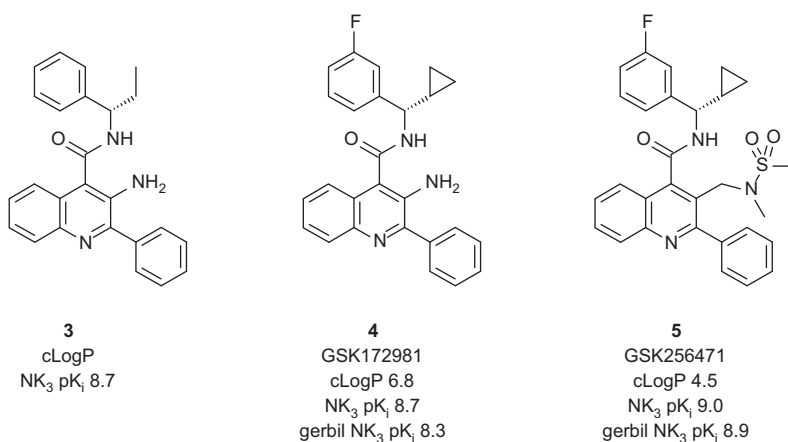
In summary, without an active transport mechanism in operation a certain minimum lipophilicity is required to facilitate permeation through the hydrophobic BBB membrane. As the size of the drug molecule increases, the lipophilicity of the molecule needs to increase to maintain the same chance of success in crossing the BBB.<sup>27</sup> However, once that minimum lipophilicity is achieved, which is defined as sufficient to provide  $P_{app} > 200$  nm/s, further increases in LogD/LogP are likely to be detrimental, increasing the extent of non-specific binding and introducing a number of additional metabolic and toxicological liabilities. Controlling the lipophilicity is also important where a short onset of action is required.<sup>10,39</sup>

An example of how controlling lipophilicity can improve brain exposure has been reported by Johnson *et al.*<sup>40</sup> In the course of a programme to identify selective muscarinic M1 agonists for the treatment of cognitive deficits associated with schizophrenia and Alzheimer's disease, compound **1** was identified (Table 18.4). This compound had a moderate cLogP of 3.5 and a whole brain:plasma ratio  $K_p$  of 5.7; however, the total unbound free drug concentrations were low at  $C_{u,br}$  2.5 nM and  $C_{u,pl}$  2.6 nM, probably due to high clearance (Table 18.4). Encouragingly, the ratio of unbound drug in brain and plasma  $K_{p,free}$  was  $\sim 1$ , indicating no active efflux in contrast with earlier analogues within the series. To address the metabolic instability, a series of compounds with reduced cLogP was prepared, resulting in the identification of compound **2** with reduced plasma and brain tissue binding ( $F_{u,pl}$ ,  $F_{u,br}$ ), lower clearance and much improved  $C_{u,br}$ . Notably the reduced cLogP of **2** (1.53 *vs.* 3.48 for **1**) led to a reduction in the whole brain:plasma ratio  $K_p$  but due to the increased  $F_{u,br}$  and reduced clearance, the overall effect was to increase the unbound concentration in brain  $C_{u,br}$ . This example highlights that strategies to lower clearance from plasma by reducing cLogP may also increase the  $F_{u,br}$ . The parameters thus work synergistically to have a beneficial impact on  $C_{u,br}$ .

Another example that highlights the importance of looking beyond whole brain levels to assess CNS exposure has been reported by Smith *et al.* at GSK.<sup>41</sup> The authors discuss the optimization of a series of neurokinin-3 (NK<sub>3</sub>) receptor antagonists starting from the lead compound **3** (Table 18.5). The first round of

**Table 18.4** Pharmacokinetic profile in rat for M1 agonists **1** and **2**.

Compound	$Cl_b^a$ ml/min/kg	$K_p$ (B:P)	$F_{u,br}$ %	$F_{u,pl}$ %	$C_{u,br}^a$ (nM)	$C_{u,pl}^a$ (nM)
<b>1</b>	85	5.7	6	20	2.5	2.6
<b>2</b>	23	1.7	39	38	261	265

<sup>a</sup>Estimated from a 3 mg/kg po dose.**Table 18.5** Exposure data generated in Sprague-Dawley rats and in gerbil cortex NK<sub>3</sub> occupancy study.

Rat			Gerbil			
Compound	Brain $C_{max}$ (ng/g) <sup>a</sup>	Dose mpk route	Mean total $C_{brain}$ ng/g (nM)	$F_{u,br}$ %	$C_{u,br}$ nM	Mean RO %
<b>3</b>	80	—	—	—	—	—
<b>4</b>	464	30 ip	2,062 (5,011)	0.7	35	60
<b>5</b>	43	10 po	61 (118)	3.3	4	61

<sup>a</sup>Sprague-Dawley male rats. Dose 3 mpk po.

optimization focused on lowering cLogP whilst maintaining potency and increasing the brain  $C_{\max}$ . Importantly, since the  $C_{\max}$  whole brain levels do not necessarily reflect the brain unbound drug concentration the authors selected the two antagonists **4** and **5** for an *ex vivo* NK<sub>3</sub> receptor occupancy study in gerbil. As can be seen in Table 18.5, despite the higher whole brain exposure seen with compound **4** the receptor occupancy (RO) at 30 mpk ip was equivalent to that seen with compound **5** at 10 mpk po. The increased potency of compound **5** and the reduced non-specific binding to brain tissue ( $F_{u,br}$  3.3% vs.  $F_{u,br}$  0.7% for **4**) compensate for the lowered whole brain level and thus predict equivalent efficacy *in vivo*. As compound **5** has the lower cLogP (4.5 vs. 6.8 for **4**) it thus has lower intrinsic risk with respect to development.

The examples above focus on lowering the lipophilicity of candidate compounds and highlight that high whole brain to plasma levels are not a prerequisite of a successful CNS drug candidate. However, high non-specific binding to brain tissue need not preclude a compound going on to become a successful drug either.<sup>42</sup> Indeed, consistent with the observation that CNS drugs have higher LogP on average than non-CNS drugs,<sup>14</sup> many commercially successful CNS therapeutics have high brain tissue binding, for example sertraline (Zoloft).<sup>42</sup> Furthermore, an example of increasing LogP (and decreasing PSA) to improve brain penetration is highlighted in Section 18.2.2.4 below.

### 18.2.2.2 Hydrogen Bonding

H-bonding has a significant impact on the CNS penetration of a molecule. Increased H-bonding potential is correlated with increased polar surface area (PSA) and has been associated with reduced BBB penetration. Leeson as well as Davis and Polli *et al.* have shown in comparative analyses of CNS and non-CNS drugs that the CNS agents have a significantly reduced number of H-bond donors (CNS mean 0.67)<sup>14</sup> and H bond acceptors (CNS mean 2.12).<sup>23</sup> Following an analysis of LogBB data for 70 compounds, Österberg showed that H-bond descriptors in combination with cLogP were sufficient to generate a BBB penetration model with good predictivity.<sup>43</sup> This work was subsequently extended to provide two simple rules for predicting LogBB values, which are indicative of good brain penetration<sup>21</sup>:

- $N + O \text{ atoms} < 5$
- $\text{cLogP} - (N + O) > 0$

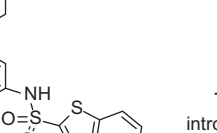
H-bonding also has an effect on the rate of permeation.<sup>15</sup> It has been shown that H-bond acidity and basicity both decrease the rate of permeation of the BBB.<sup>44</sup> Studies in the PAMPA assay devoid of efflux transporters suggest that increased H-bonding potential can lower passive permeability;<sup>15</sup> however, it is likely that the role of H-bonding is two-fold since increased H-bonding potential also increases the risk that efflux *via* P-gp will occur. As a consequence, the reduction of H-bond donors and acceptors is often a successful

strategy in the optimization of drugs targeting the CNS. Pajouhesh and Lenz have proposed the frequently quoted guidelines of H-Bond donors <3; H-bond acceptors <7; and total H-bonds <8 as attributes of a successful CNS drug candidate.<sup>24</sup>

Johnson *et al.* have described the successful optimization of the poor brain penetrant 5-HT<sub>6</sub> antagonist SB-271046 **6** by focusing on hydrogen bond count and the number of rotational bonds.<sup>45</sup> SB-271046 **6** had good oral bioavailability; however, its brain:plasma ratio  $K_p$  was 0.05 and the compound was shown to be an efflux substrate of P-gp. To improve brain penetration compounds such as **7** and **8** were prepared, where the acidic NH donor group of sulfonamide has been removed by cyclization onto the adjacent phenyl ring (Table 18.6). This also reduces the overall flexibility of the molecule, which is predicted to further benefit BBB permeability (Section 18.2.2.6).

Removal of the acidic NH and imposing a conformational constraint as in analogues **7a–b** afforded potent 5-HT<sub>6</sub> ligands; however, these compounds suffered from high *in vivo* blood clearance. Resolution of this issue afforded compound **7c**, which was shown to have reduced efflux liability in an MDR1-MDCK assay. Alternative cores such as compounds **8a–b** were also investigated and in this case the removal of the additional H-bond donor in **8a** to afford **8b** leads to an increase in the brain:plasma ratio ( $K_p$  2.6 vs. 0.7). To confirm that the elevated  $K_p$  values were indicative of improved  $C_{u,br}$  compounds, **7c** and **8b** were selected for evaluation in a rat *ex vivo* binding assay. Compounds **7c** and **8b** had ED<sub>50</sub> of 3 and 5 mpk po, respectively. In comparison the initial compound SB-271046 **6** was significantly less potent with

**Table 18.6** 5-HT<sub>6</sub> binding affinity of compounds **7a–c** and **8a–b**.

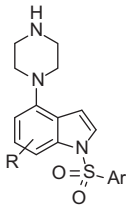


SB-271046 **6**

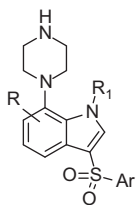
remove acidic  
H-bond donor

introduce conformational  
constraint

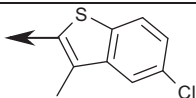
→



**7a–c**



**8a–b**

Compound	Ar	R	R <sup>1</sup>	pK <sub>i</sub>	rat Cl <sub>b</sub> ml/min/kg	K <sub>p</sub>
<b>7a</b>		H	–	8.5	–	–
<b>7b</b>	C <sub>6</sub> H <sub>4</sub> (3-Cl)	H	–	9.6	–	–
<b>7c</b>	C <sub>6</sub> H <sub>4</sub> (3-Cl)	5-Cl	–	8.6	44	3
<b>8a</b>	C <sub>6</sub> H <sub>4</sub> (3-Cl)	H	H	9.5	41	0.7
<b>8b</b>	C <sub>6</sub> H <sub>4</sub> (3-Cl)	H	Me	8.6	34	2.6

an ED<sub>50</sub> of 11 mpk po. This example demonstrates that when  $K_p$  values are  $\ll 1$  and P-gp efflux has been identified,  $K_p$  measurements can be effectively used early in the screening cascade as a quick way to prioritize compounds with reduced P-gp liability for further study.

Intra-molecular H-bonding can also be exploited to improve the BBB permeability by masking the polarity of the H-bond functional group in the molecule. This also contributes to reduced molecular flexibility, which further benefits brain exposure (*vide infra* Section 18.2.2.6).<sup>59,60</sup>

### 18.2.2.3 Polar Surface Area

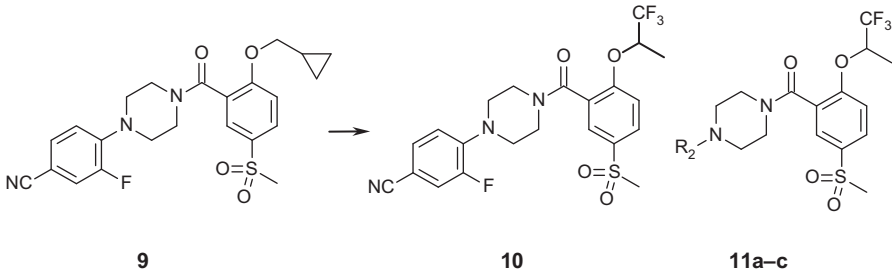
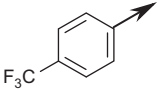
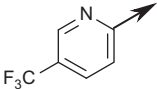
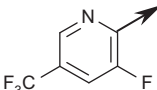
Polar surface area (PSA) is often described as a surrogate measure of hydrogen bonding capacity and polarity. Early computational QSAR studies of LogBB data by van de Waterbeemd and Kansy identified that PSA was a key descriptor in determining the extent of BBB penetration.<sup>18</sup> The initial studies were confirmed when larger data sets were analyzed;<sup>19,20,44,46–47</sup> however, many of these studies have produced QSAR equations that rely on access to special computational software and are not easily interpreted outside the cyber environment by medicinal chemists. In analyses of non-CNS *versus* CNS drugs the groups of both Kelder and van de Waterbeemd translated their results into guidelines for optimal PSA.<sup>46,20</sup> Van de Waterbeemd proposed a PSA cut-off of  $<90 \text{ \AA}^2$  whilst Kelder proposed the more stringent cut-off of  $<60\text{--}70 \text{ \AA}^2$ . The effect of PSA on the extent of brain penetration could be due to changes in brain tissue binding or differences in permeability through the BBB, but in reality PSA probably influences both. In an analysis of permeability LogPS data for 23 compounds Liu *et al.* demonstrated that PSA is one of three key descriptors that can be used to generate a linear model of brain permeation.<sup>38</sup> However, PSA alone has been shown to have only modest predictive power even for a simpler data set such as Caco-2 permeability.<sup>33</sup> Egan's "confidence ellipse" based on ALogP98 and PSA, which was used at Pharmacopeia to predict Caco-2 permeability, is perhaps one of the simplest to apply composite models of PSA that can be used to predict permeability.<sup>34</sup>

Pinard at Roche<sup>48</sup> has described the discovery of the selective GlyT1 inhibitor RG1678, which has demonstrated efficacy in a phase II clinical study in schizophrenic patients. Using their proprietary SAR analyzer (ROSARA) they identified that PSA and cLogP were the sole descriptors required to predict brain penetration within the series. The starting point for their work was lead compound **9**, which had fairly low brain penetration ( $K_p \sim 0.1$ ) and unacceptable hERG potency (IC<sub>50</sub> 0.6  $\mu\text{M}$  patch clamp assay) (Table 18.7). The optimization challenge was thus to balance the conflicting requirements of low enough PSA for acceptable brain permeation and sufficiently high PSA to reduce hERG liability. The authors commented that they could correlate improvements in efficacy in their *in vivo* model with improvements in  $C_{\text{brain}}$  for this series (implying  $F_{\text{u,brain}}$  remained relatively constant between analogues) and thus used this measure in combination with  $K_p$  to screen compounds.



The trifluoroisopropoxy group as in derivative **10** proved a favoured substituent despite minimal changes in PSA and cLogP, improving the brain exposure and *in vivo* efficacy without being detrimental to hERG. Replacement of the nitrile group with a trifluoromethyl group afforded **11a** (cLogP 3.92, PSA 59 Å<sup>2</sup>) with lower PSA and higher cLogP than the lead **9** (cLogP 2.64, PSA 80 Å<sup>2</sup>) and an improved K<sub>p</sub> ratio. Addition of the pyridyl nitrogen in **11b** to decrease the hERG affinity was detrimental to *in vivo* efficacy. To address this, the authors followed a well-documented strategy of fluorine insertion to improve brain penetration;<sup>49</sup> compound **11c** maintained the selectivity over the hERG channel with minimal changes in physicochemical properties and potency compared with **11b** and yet showed significantly improved *in vivo* efficacy and K<sub>p</sub>. Resolution of **11c** identified the (*S*)-enantiomer as the eutomer and this compound was selected for progression into the clinic as **RG1678** and is still under investigation following positive phase II results (see Chapter 4) (Table 18.7).

**Table 18.7** GlyT1 uptake inhibition, pharmacokinetic and safety profile of compounds **9**, **10** and **11a–c**.

							
Compound	R <sup>1</sup>	GlyT1 EC <sub>50</sub> μM	hERG IC <sub>50</sub> μM	cLogP	PSA Å <sup>2</sup>	<i>in vivo</i> efficacy mpk <sup>a</sup>	Mouse K <sub>p</sub>
<b>9</b>	—	0.016	0.6	2.64	80	3.0	0.10
<b>10</b>	—	0.044	3.0	2.77	80	1.0	0.25
<b>11a</b>		0.03	10	3.92	59	2.0	1.10
<b>11b</b>		0.037	20	2.82	69	5.0	0.20
<b>11c</b>		0.040	20	3.00	69	1.0	0.50
<b>(S)-11c</b> <b>(RG1678)</b>		0.030	17	3.00	69	0.5	0.5

<sup>a</sup>Reversal of L-687,414-induced hyperlocomotion in mouse.

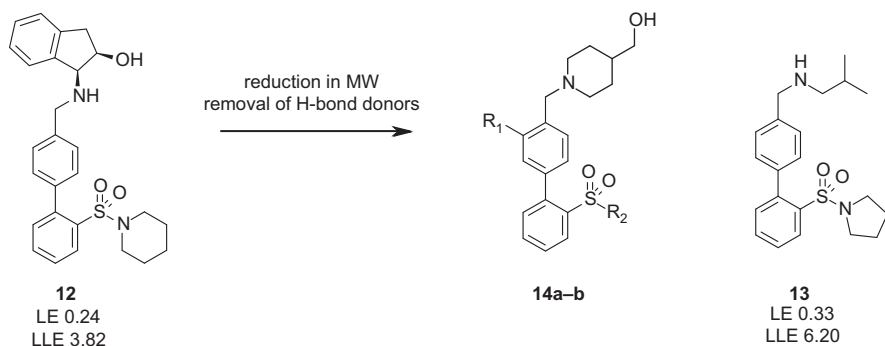
#### 18.2.2.4 Molecular Weight

The difference between the average molecular weight of CNS therapeutics and non-CNS drugs is well documented.<sup>23</sup> The mean MW of CNS drugs (1983–2002) is 310 with a median of 305–307<sup>23,28</sup> in comparison to a mean MW for oral therapeutics (including CNS drugs) of 377 and a median of 357.<sup>23</sup> In an analysis of brain capillary permeability coefficient (*P*) measurements in ether-anaesthetized rats, Levin suggested that there was an MW cut-off for passive brain permeability somewhere between MW 400 and 657<sup>50</sup> and following an analysis of CNS *versus* non-CNS drugs van de Waterbeemd proposed that the value should be MW <450.<sup>20</sup> Lowering molecular weight will often have co-incidental beneficial effects on other parameters such as PSA and cLogP and it is thus a frequently used strategy to optimize CNS penetration. This is usually accompanied by close monitoring of the ligand binding efficiency (LE)<sup>51</sup> and lipophilic ligand binding efficiency (LLE).<sup>52</sup> The minimum pharmacophore can thus be identified as the basis for further optimization.

An example of this strategy where close monitoring of physicochemical properties, LE and LLE, was used during optimization has been reported by Verhoest *et al.* at Pfizer.<sup>53</sup> The goal was identification of a CNS-penetrant selective  $\kappa$ -opioid antagonist as a development candidate for depression and substance abuse. The starting point for their work was the HTS hit **12**. This compound had high MW (462 Da) and cLogP (4.13) for a CNS lead compound. Furthermore, it displayed low selectivity over the  $\mu$ -opioid receptor and had undesirable ADME properties with high human liver microsomal clearance (>300 (ml/min)/kg) and suspected P-gp efflux (MDR efflux ratio 2.56). However, since the synthesis of analogues was trivial and highly amenable to library synthesis, the Pfizer group elected quickly to evaluate the scope for optimization by setting target constraints for synthesis of 0–1 hydrogen bond donors, MW <425 and cLogD <3 commensurate with preferred CNS drug properties. Given the 3-dimensional format of the array some compounds fell outside these limits but the majority were compliant (Table 18.8).

The library successfully identified the peripheral aryl ring in the indolene in **12** as surplus to requirements since it could be replaced by a number of smaller heterocyclic and aliphatic amines, which reduced the global molecular weight, exemplified by the *sec*-butylamine **13**. The two analogues **14a** (MW >429, cLogD >3) and **14b** (MW >429, H-bond donors >1) (Figure 18.8), which fell outside the proposed physicochemical constraints, afforded sub-optimal compounds with respect to clearance (**14a** >304 ml/min/kg) or MDR efflux ratio (**14b**, ER 14.0).

Following *in vivo* evaluation of selected analogues compound **13** was selected for progression. The ligand efficiency and lipophilic ligand efficiency were significantly improved relative to the starting hit **12** (LE improved from 0.24 to 0.33 and LLE improved from 3.82 to 6.20). Compound **13** showed good brain penetration in rats (AUC<sub>0–4h</sub> free brain/free plasma ~1, AUC<sub>0–4h</sub> CSF/free plasma 1.2, AUC<sub>0–4h</sub> CSF/free brain ~1.4) with no evidence of active efflux.

**Table 18.8**  $\kappa$ - and  $\mu$ -opioid binding affinities and *in vitro* DMPK properties of compounds **12**, **13** and **14a–b**.

Compound	R <sup>1</sup>	R <sup>2</sup>	Kappa <sup>a</sup> K <sub>i</sub> nM	Mu <sup>a</sup> K <sub>i</sub> nM	HLM <sup>b</sup> Cl <sub>int</sub>	MDR efflux ratio	cLogD	MW
<b>12</b>	–	–	9	21	> 300	2.56	4.13	462
<b>13</b>	H		3.0	64	27.6	1.96	2.32	372.5
<b>14a</b>	F		8.8	112	> 304	1.50	3.58	460.6
<b>14b</b>	H		1.1	32.0	163	14.0	1.48	472.6

<sup>a</sup>Human  $\kappa$ - and  $\mu$ -binding K<sub>i</sub>.<sup>b</sup>Human liver microsome intrinsic clearance (ml/min)/kg.

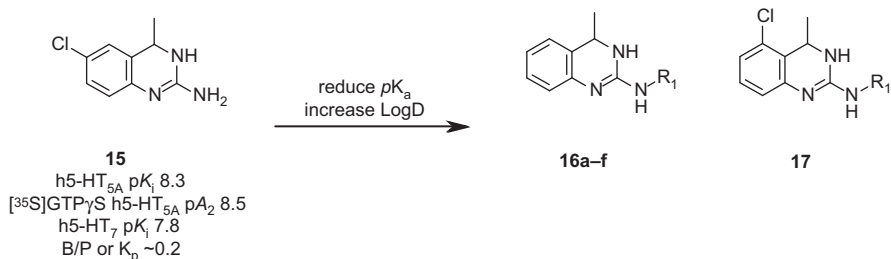
### 18.2.2.5 pK<sub>a</sub> and Ionization State

Pfizer conducted an analysis of 119 marketed CNS drugs and 118 Pfizer CNS clinical candidates and observed that the mean pK<sub>a</sub> is 8.4, reflecting the well-acknowledged fact that the majority of CNS drugs have a basic centre.<sup>28</sup> There are two schools of thought as to whether this reflects an inherent benefit of compound basicity for brain exposure or merely the fact that the data set is biased since many of the currently prescribed CNS drugs target monoamine receptors or transporters that have a ligand pharmacophore containing a basic centre. Pajouhesh and Lenz have proposed the optimal pK<sub>a</sub> range for CNS drugs to be pK<sub>a</sub> 7.5–10.5<sup>24</sup> and, although the lower limit would now be considered overly conservative, the upper limit is consistent with more recent analysis.<sup>28</sup> P-glycoprotein efflux probably contributes to the upper pK<sub>a</sub> limit since highly basic molecules are associated with P-gp substrate liability (*vide infra*). In a review of P-gp data for 1,000 compounds Petrauskas proposed a cut-off of pK<sub>a</sub> < 8 for compound design to avoid

possible P-gp interaction, although many CNS drugs including the SSRIs and tricyclics exceed this criterion.<sup>22</sup> The lower  $pK_a$  limit is probably a combination of enhanced P-gp liability for acids<sup>22</sup> and the inherently reduced passive permeability for acids and zwitterions on average relative to neutral and basic molecules.<sup>26</sup>

A classical approach to optimize CNS compounds is to maintain the  $pK_a$  of ionizable groups in the molecule within the limits proposed by Pajouhesh and Lenz (7.5–10.5). This strategy has been successfully applied at Hoffman-La Roche to increase the brain penetration of a series of cyclic guanidine dual 5-HT<sub>5A</sub>/5-HT<sub>7</sub> ligands.<sup>54</sup> The starting lead **15** had modest brain penetration, with a brain-to-plasma ratio of  $K_p = 0.2$ . This was attributed to the high  $pK_a \sim 10$  of the amine and the low  $cLogD \sim 0$ . Two strategies were applied to improve the brain penetration, adding lipophilicity and lowering the  $pK_a$ . Initial SAR was carried out on the synthetically more accessible unsubstituted aromatic as in **16a–f**. As can be seen from Table 18.9 the addition of lipophilicity alone by introducing alkyl chains into the R<sup>1</sup> group (**16b–c**) had only modest effects on LogD and  $pK_a$  given the strongly basic nature of the guanidine group. The more successful approach was to lower the  $pK_a$  by adding fluoroethyl R<sup>1</sup> substituents. Although the monofluoro- and trifluoro-ethyl analogues lost potency at 5-HT<sub>5A</sub> the difluoroethyl compound **16e** was only 2.3-fold less potent than parent compound **16a**. Re-introduction of the preferred electron withdrawing chloro-substituents as in **17** further lowered the  $pK_a$ , increased LogD and afforded a potent 5-HT<sub>5A</sub> antagonist with physicochemical properties in line with those suggested for CNS compounds. Pharmacokinetic assessment of compound **17** showed an

**Table 18.9** 5-HT<sub>5A</sub> affinity and physicochemical properties of compounds **15–17**.



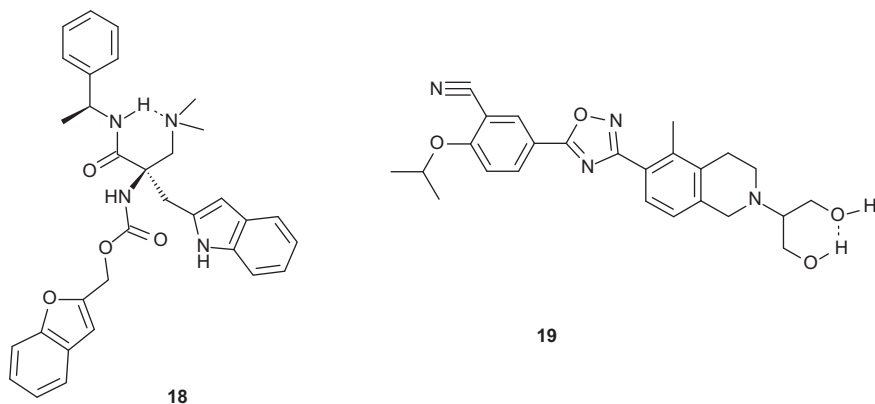
Compound	R <sup>1</sup>	5-HT <sub>5A</sub> $K_i$ nM	5-HT <sub>7</sub> $K_i$ nM	$pK_a$	Log D <sub>7.4</sub>
<b>16a</b>	H	38	—	10.5	−1.4
<b>16b</b>	Me	103	—	10.6	−0.6
<b>16c</b>	Et	196	—	10.8	−0.9
<b>16d</b>	CH <sub>2</sub> CH <sub>2</sub> F	397	—	—	—
<b>16e</b>	CH <sub>2</sub> CHF <sub>2</sub>	87	—	9.7	−0.2
<b>16f</b>	CH <sub>2</sub> CF <sub>3</sub>	488	—	9.2	0.5
<b>17</b>	CH <sub>2</sub> CHF <sub>2</sub>	10.1	33.3	8.9	1.5

improved brain-to-plasma ratio ( $K_p \sim 4$ ) with a 20-fold increase in whole brain levels corresponding to  $>1\mu\text{M}$  brain concentrations at 0.8–4 h (6.6 mpk po) although data supporting a concomitant improvement in  $C_{u,br}$  were not reported.

#### 18.2.2.6 Flexibility and Number of Rotatable Bonds

Veber *et al.* have highlighted the importance of rotational bond count in predicting rat oral bioavailability and permeation rates in an artificial membrane permeation assay, demonstrating that increased molecular flexibility has a negative impact on the process of passive permeation.<sup>15</sup> Consistent with the supposition that such effects would have a more profound influence at the BBB, Leeson and Davis have shown that the average rotational bond count of oral CNS drugs (mean 4.7, median 4.5) within a data set of oral drugs (1983–2002 NCE list) is reduced relative to the global average across all therapeutic areas (mean 6.4, median 6).<sup>23</sup> This has led to a proposed guideline of rotatable bond count  $<8$  as an attribute of a successful CNS drug candidate.<sup>24</sup>

Rigidification of a molecule often involves changes to the hydrogen bond count, molecular weight, lipophilicity and PSA *via* the introduction of new ring systems, hence is it rare to find reported examples where this approach has been used in isolation. However, it is a commonly used strategy wherein both covalent<sup>45,55–57</sup> (see Section 18.2.2.2) and non-covalent (intra-molecular H-bonding)<sup>59,60</sup> manifolds have been invoked. For example, intra-molecular H-bonding and the resulting reduction in polarity and flexibility have been postulated to contribute to the improved brain exposure of the NK<sub>1</sub> antagonist PD 174424 **18**<sup>58</sup> and S1P<sub>1</sub> agonist **19**<sup>59</sup> (Figure 18.3).



**Figure 18.3** Examples of intra-molecular bonding to improve brain exposure.

### 18.2.3 P-glycoprotein Efflux at the BBB

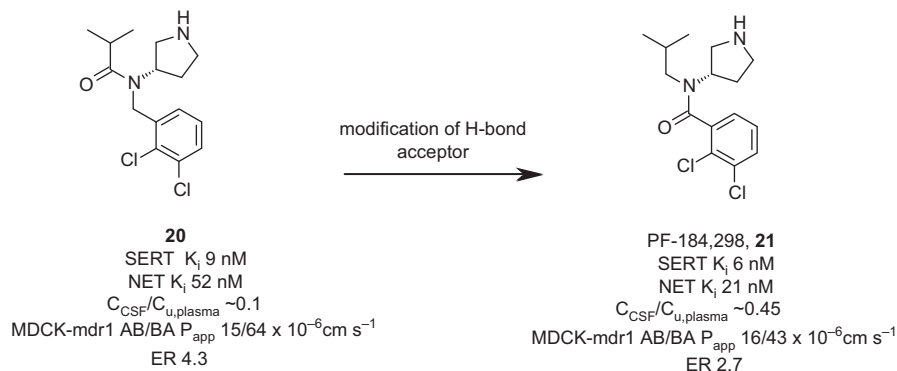
Limited brain exposure due to efflux of a compound by P-glycoprotein (P-gp) at the BBB is a very common issue in CNS drug discovery. P-gp (MDR1, ABCB1) is a membrane protein of the ATP-binding cassette transporter superfamily, which is expressed in a variety of human tissues in addition to its presence at the BBB. It acts as a gatekeeper for tissue exposure and provides a natural defence mechanism against xenobiotics. As such, P-gp has evolved to be highly promiscuous, recognizing a large variety of molecules.

Attempts to predict whether a compound will be a P-gp substrate based on structure have looked at either pharmacophore models using known substrates<sup>60</sup> or physicochemical property analyses.<sup>61–63</sup> However, both approaches have flaws; the pharmacophore generation is hampered by the fact that there are several binding domains within P-gp and without detailed experimental data for each compound any models generated obfuscate the distinction, and the physicochemical property approach is equally challenging since it is unlikely to have the subtlety to account for micro-phenomena such as protein SAR. The recent publication of low-resolution crystal structures of P-gp and the generation of homology models allow for the possibility of ligand docking approaches; however, this is not a routinely reported approach to date.<sup>64</sup> The ready availability of *in vitro* models of P-gp efflux such as the MDR1-MDCK and Caco-2 cell lines mean that the utility of predictive models is perhaps mitigated.

P-gp is arguably one of the most studied antitargets in medicinal chemistry and as such there are a number of reviews covering research into P-gp.<sup>65,66</sup> This chapter will thus touch only briefly on the predictive approaches mentioned above and focus instead on examples of overcoming P-gp efflux at the BBB from the CNS literature. Since many of the data sets used to provide the physicochemical property guidelines in Section 18.2.2 incorporate the effects of P-gp and related active transporters (for example, LogBB,  $K_p$ , Caco-2, MDR1-MDCK data sets), following those guidelines will help reduce the risk of transporter efflux.

To emphasize the more significant effect of P-gp on CNS drug exposure relative to other therapeutic areas, Polli *et al.* have shown that P-gp efflux differentiates CNS and non-CNS drugs.<sup>14</sup> The CNS drug set had a three-fold lower incidence of P-gp mediated efflux suggesting the enhanced sensitivity of brain tissue to substrate activity at P-gp. Recently a rule of 4 has been formulated to predict P-gp efflux liability,<sup>22</sup> where compounds with molecular weight <400, total number of nitrogen and oxygen atoms <4 and basic  $pK_a$  <8 are considered unlikely to be P-gp substrates. A significant number of CNS-active molecules exceed one of these criteria; however, this rule of 4 is a useful indicator of strategies that can be applied to reduce efflux by P-gp. Reducing molecular weight, lowering  $pK_a$  and reducing the number of heteroatoms and H-bond donors in a molecule have all been demonstrated to reduce P-gp activity in different chemical series.

Whitlock *et al.* have tackled the issue of efflux from the CNS by P-gp during the discovery of the dual serotonin and noradrenaline reuptake inhibitor



**Figure 18.4** Optimization of P-glycoprotein efflux for dual serotonin and norepinephrine reuptake inhibitors.

(SNRI) clinical candidate PF-184,298 **21**.<sup>67,68</sup> The starting compound **20** exhibited many physicochemical properties consistent with CNS space including low MW, but showed no efficacy in the *in vivo* model due to poor CNS penetration ( $C_{CSF}/C_{u,plasma} \sim 0.1$ , a surrogate measure of  $K_{p,free}$ ) with a significant degree of efflux in the MDR1-MDCK cell line (ER 4.3) (Figure 18.4). Although the  $pK_a$  of the secondary amine was high ( $pK_a$  9.4), it is consistent with many molecules targeting the monoamine transporters and is a key part of the pharmacophore. Thus, rather than address global physicochemical properties the group at Pfizer elected to adjust the H-bonding capability of **20** to disrupt P-gp recognition. The subtle change in **21** wherein the carbonyl of the amide has been moved to the benzylic position was enough to reduce the P-gp efflux (ER 2.7) and afford improved brain exposure ( $C_{CSF}/C_{u,plasma} \sim 0.45$ ) and efficacy in the preclinical *in vivo* model.

Hong *et al.* at Lundbeck have reported the strategies they employed to lower the P-gp efflux liability in a series of indole azetidine MCHR1 antagonists.<sup>69</sup> Initial compound **22** was confirmed as a P-gp substrate with poor inherent permeability *via* a number of methods: the efflux ratio in the MDR1-MDCK permeability assay was 144 with low  $P_{app}$  A-B  $0.19 \times 10^{-6} \text{ cm s}^{-1}$ , in P-gp KO mice a 6–8 h window is required to reach high brain levels, and the brain/plasma ratio over 8 h was substantially improved in P-gp KO mice (B/P 2.7) *versus* wild-type (B/P 0.3) (Table 18.10).

Hong *et al.* employed a design strategy focused on lowering the  $pK_a$ , reducing the number of heteroatoms, removing the hydrogen bond donor and reducing conformational flexibility. The first compound **23a** (Table 18.11) showed the beneficial effect of lowering  $pK_a$  on the efflux ratio; however, efflux remained unacceptably high. Introducing conformational rigidity by removing the flexible hydroxymethyl linker as in **23b** also improved the efflux ratio. Combining these two effects and also removing the H-bond donor as in **23c** further improved the efflux ratio. However at ER > 3 compound **23c** is still



**Table 18.10** MCHR1 binding affinity and *in vitro* permeability data for compounds **22**, **23a–c**, **24a–b**.

Compound	$R_1$	$R_2$	$R_3$	$rMCH\ K_i$ (nM)	$P_{app}^{A-to-B}$ ( $\times 10^{-6}\text{ cm s}^{-1}$ )	Efflux ratio	$pK_a$
<b>22</b>	—	—	—	11	0.2	144	8.7
<b>23a</b>	PhCH <sub>2</sub> O	H	MeO(CH <sub>2</sub> ) <sub>2</sub>	5.2	0.4	88	8.2
<b>23b</b>	4-Cl-Ph	H	Me	7.8	0.9	60	8.7
<b>23c</b>	4-F-Ph	Me	F-(CH <sub>2</sub> ) <sub>2</sub>	9.0	7.1	6.4	7.4
<b>24a</b>	PhCH <sub>2</sub> O	—	Me	2.5	2.6	14	8.6
<b>24b</b>	4-Cl-Ph	—	F-(CH <sub>2</sub> ) <sub>2</sub>	6.5	28.9	1	6.9

**Table 18.11** Plasma and brain exposure of MCHR1 antagonists **25** and **26** in DIO mouse model after 10 mpk po.

	Plasma AUC (ng h/ml)	Brain AUC (ng h/ml)	Plasma $T_{1/2}$ (h)	Brain $T_{1/2}$ (h)	Plasma $C_{max}$ (ng/ml)	Brain $C_{max}$ (ng/ml)
<b>25</b>	164	—	2.3	—	63	—
<b>26</b>	8,762	59,057	3.1	2.9	1,183	12,140

deemed a P-gp substrate. Switching to a dihydroindole core afforded measurable benefits since the isostere of **22**, compound **24a**, showed a ten-fold reduction in the ER. Adding the favoured substituents from the indole series to reduce conformational flexibility and the amine  $pK_a$  afforded compound **24b**, which shows a vastly improved inherent permeability as well as being devoid of P-gp efflux in the MDR1-MDCK assay.

### 18.2.4 Increasing Systemic Exposure to Increase Brain Exposure

When systemic exposure is low due to high blood clearance, then it is often prudent to address this issue in advance of implementing strategies to target any CNS-specific issues such as low BBB permeability or low  $K_p$ . This is because strategies to increase BBB passive permeability or  $K_p$  often increase blood clearance and raise non-specific binding, leading to an overall decrease in  $C_{u,brain}$  even though the rate of permeability of the BBB may be improved. Conversely, as seen in the example from Johnson *et al.* in Section 18.2.2.1, strategies that attempt to lower blood clearance may lower the  $K_p$ , but as long as passive permeability is not significantly compromised this may be sufficient to increase the  $C_{u,brain}$ . Simply put, if  $C_{u,plasma}$  is increased due to an increase in  $C_{plasma}$ , then at steady state (with free equilibration across the BBB) this will be sufficient to afford a concomitant increase in  $C_{u,brain}$ . An example that illustrates this point comes from the group of Vasudevan *et al.* at Abbott.<sup>70</sup> They reported the identification of aminopiperidine indazoles as MCHR1 antagonists. Within the series a drastic change in the brain exposure was seen when the N-1 nitrogen was acylated, which was attributed to high clearance from plasma. Compound **25** was rapidly cleared from the plasma with concentrations declining below the limits of detection within 4 hours; moreover, brain levels were below levels of detection at all time points. In contrast, the analogue **26** with an additional H-bond donor but much reduced blood clearance afforded excellent plasma and brain exposure and, interestingly, was preferentially partitioned into brain.

## 18.3 Safety and Tolerability: Common Challenges in CNS Drug Discovery

Although the introduction of sophisticated DMPK assays and translational models has significantly reduced the risk of pharmacokinetics-related failure of drug candidates in clinical development, the pharmaceutical industry still faces approximately 30% attrition of drug candidates due to safety and tolerability findings.<sup>71</sup> Further, 10% of new chemical entities (NCEs) show serious side-effects or adverse drug reactions (ADRs) after market launch, resulting in “black box” warnings, label changes or market withdrawal.

The diversity of mechanisms that give rise to toxicity presents a major challenge to the development of predictive preclinical models to guide prioritization and optimization efforts in drug discovery. In recent years there has been considerable interest in analyzing the physicochemical properties of drug candidates and marketed drugs as an approach to delineate general guidelines that could help design molecules with improved chances of success in drug development. The top level message emerging from these studies is that high physicochemical properties are associated with increased risks of not only poor DMPK properties,<sup>72</sup> but also poor safety and tolerability profiles.<sup>51,73</sup>

In particular, lipophilicity has emerged as the most critical physicochemical parameter and a robust predictor of clinical success. Its impact on drug potency, pharmacokinetics and toxicity has been known for many years.<sup>74–76</sup>

An analysis of the Cerep BioPrint database of drugs and reference compounds clearly demonstrated that overall promiscuity, defined as the number of assays in which a compound shows > 50% inhibition at 10  $\mu$ M concentration, is largely controlled by lipophilicity and ionization state.<sup>51</sup> Bases and quaternary bases are notably more promiscuous than acids, neutral compounds or zwitterions, but in all ionization classes promiscuity correlates positively with clogP.<sup>51</sup> Furthermore, an analysis of relationships between physicochemical properties and safety inferred from *in vivo* safety data for 245 preclinical compounds at Pfizer has indicated significantly increased risk of toxic events for basic nitrogen-containing compounds with clogP > 3 and PS < 75 Å.<sup>77</sup> Interestingly, this “high-risk” profile is very similar to properties that are considered optimal for CNS drugs as described above (clogP 2.8; PSA 44.8; pK<sub>a</sub> 8.4),<sup>28</sup> which highlights challenges faced by medicinal chemists working on CNS targets.

Some of the commonly reported antitargets in CNS drug discovery and their associated side-effects are listed in Table 18.12.<sup>78</sup>

In 1997 drug-induced valvular heart disease (VHD) led to the withdrawal of the appetite suppressant fenfluramine (Pondimin). Several years later it was found that its active metabolite norfenfluramine is a potent 5-HT<sub>2B</sub> receptor agonist, a property shared with other VHD-associated drugs, such as the antimigraine methysergide and dihydroergotamine. This led to the conclusion that activation of the 5-HT<sub>2B</sub> receptor, highly expressed in heart valves, is responsible for VHD.<sup>79</sup> The adrenergic  $\alpha_{1a}$  receptor mediates the relaxation of the vascular muscle tone and is important for blood pressure regulation. It has been reported as an antitarget responsible for cardiovascular side-effects such as hypotension, dizziness and fainting spells in a number of CNS drug candidates.<sup>80,81</sup> Similarly, parasympathetic effects mediated by muscarinic M2 and M3 receptor activation, including bradycardia, increased gut motility and salivation, have hampered numerous psychiatric drug discovery efforts.<sup>82</sup>

CYPs are the major drug-metabolising enzymes, responsible for over 70% of the total metabolism. Many drugs are known to increase or decrease the activity of various CYP isoforms either by inducing their biosynthesis (CYP induction) or by directly inhibiting the enzyme (CYP inhibition). These changes in CYP enzyme activity may affect another drug's exposure, a phenomenon

**Table 18.12** Antitargets and associated side-effects.

<i>Antitarget</i>	<i>Associated side-effects</i>
Serotonin 5-HT <sub>2B</sub> agonist	Valvular heart disease
Adrenergic $\alpha_{1a}$ antagonist	Othostatic hypotension, dizziness and fainting spells
Muscarinic M2, M3 agonist	Bradycardia, increased gut motility, extensive salivation
Cyp inhibitors/inducers	Drug–drug interactions
hERG blocker	QT prolongation, which can lead to ventricular fibrillation and death

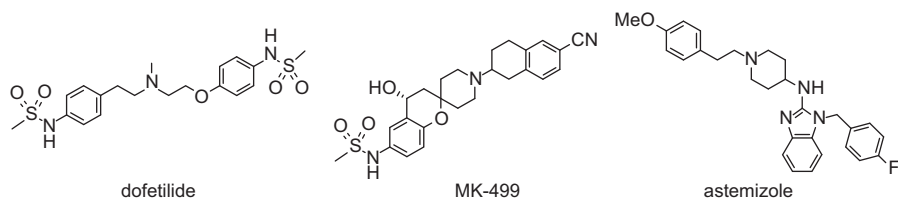
referred to as drug–drug interaction (DDI) and a major cause of adverse drug interactions (ADRs) in the clinic. For example, a drug that inhibits the CYP-mediated metabolism (CYP inhibitor) may result in accumulation of another drug within the body to toxic levels, whilst CYP inducers can increase the clearance of another drug and consequently diminish its effect. In an effort to assess and address the risk of DDIs at early stages in drug discovery, high-throughput inhibition assays for major CYPs involved in metabolism of xenobiotics (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were adopted across the industry as a part of the standard safety assessment package.<sup>78</sup> Furthermore, to minimize the risk of a drug becoming a “victim” of DDI, general preference in optimization is given to compounds that are metabolized by multiple rather than single CYP isoforms. It is particularly advisable to avoid polymorphic CYPs, such as CYP2D6.<sup>83</sup> More than 40 variants have been identified to date, showing a full spectrum of enzymatic activities, from inactivated to reduced to increased catalytic activity. For this reason drugs that are metabolized primarily by CYP2D6 show a greater risk of adverse drug reactions (ADRs) or poor efficacy.

In this section we will discuss in more detail two additional challenges highly prominent although not specific amongst the CNS programs, namely hERG selectivity and phospholipidosis.

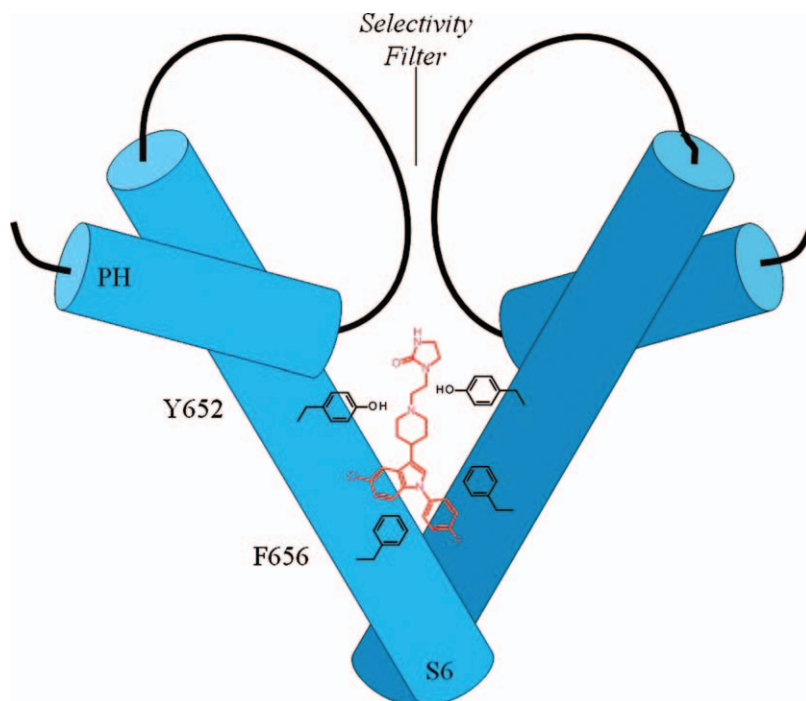
### 18.3.1 Optimization of hERG Selectivity

A number of medicines, including CNS drugs such as antipsychotic sertindole, have been labelled with “black- box” warnings or withdrawn from the market due to prolongation of the QT interval, a length of time between the start of the Q wave and the end of the T wave on an electrocardiogram. This condition is widely associated with increased risk of *Torsades de Pointes* (TdP), a ventricular tachyarrhythmia that can degenerate into ventricular fibrillation and sudden death.<sup>84</sup> The QT interval prolongation has been linked to a drug interaction with a cardiac potassium channel, a product of the human ether-a-go-go related gene (hERG), which plays a critical role in the repolarization of the cardiac myocyte action potential.<sup>85,86</sup> Consequently, throughout the pharmaceutical industry significant efforts have been invested in quantifying drug–hERG interaction. Currently a widely accepted screening cascade designed to preclinically assess hERG-related risks starts with a routine binding assay, based on displacement of radio-labelled binders such as dofetilide, MK-499 or astemizol, followed up by automated and/or manual patch clamp electrophysiology in cells expressing hERG. Key compounds are further evaluated *ex vivo* in the dog purkinje fibres assay and *in vivo*, most frequently in a dog model (Figure 18.5).<sup>87</sup>

Several homology models of the open and closed states of the hERG channel, derived from bacterial potassium channels, have been published.<sup>88,89</sup> Most drugs that inhibit hERG are believed to do so by binding to residues lining the large lipophilic central cavity of the channel. Early mutagenesis studies indicated that the aromatic residues F656 and Y652 of the channel S6



**Figure 18.5** Potent hERG blockers: commonly used radio-labelled ligands in binding assays.



**Figure 18.6** Schematic depiction of the putative interactions between sertindole and the hERG channel based on homology modelling of the closed state.<sup>89</sup> The inner helices (S6) and loops extending from the pore helices to the selectivity filter form the inner cavity and the ligand-binding site. For clarity, S6 and pore helix domains of only two subunits of the tetrameric channel are shown (longitudinal view with respect to the cell membrane).

domain are important for interactions with hERG blockers such as sertindole, an atypical antipsychotic that was withdrawn from the market in 1998 following concerns over the increased risk of sudden death from QTc prolongation (Figure 18.6).<sup>89</sup> More detailed analysis of the roles of F656 and Y652 indicate that at position 652 an aromatic residue is required, leading to the

hypothesis that Y652 participates in  $\pi$ -cation type interactions with basic amine containing hERG ligands and presumably  $\pi$ -stacking interactions for neutral hERG ligands. In the case of position 656, hydrophobic surface area was found to be the key determinant, suggesting F656 participates in hydrophobic interactions.<sup>90</sup> Strategies that disrupt the ability of a ligand to interact with F656 and Y652 have been proposed as a possible means to remove hERG activity.<sup>91</sup>

In 2005, based on new data, sertindole was reintroduced for restricted use in clinics, with strong safeguards including extensive contraindications and warnings for patients at risk of cardiac dysrhythmias. Development of Melanin Concentrating Hormone Receptor-1 (MCHR-1) antagonists for the treatment of obesity and mood disorders is another intensely pursued CNS research area in which progress has been severely affected by hERG-related issues. Despite the large number of drug discovery programs and promising preclinical validation data, only a very small number of these candidates progressed into the clinic, mostly due to cardiovascular risk related to hERG binding.<sup>92,93</sup> This may not be surprising considering a common pharmacore shared by MCHR-1 and hERG binders: a positively charged group and at least one distal aromatic/hydrophobic region. It is widely accepted that the amino group is critical for MCHR binding due to its interaction with conserved Asp123 deep within the receptor transmembrane helix (TM)-3.<sup>94</sup> With respect to hERG binding, it is hypothesized that the positively charged amino group may participate in a cation- $\pi$  interaction with the Tyr-652, whilst the distal aromatic/hydrophobic moieties may interact with Phe-656 residue of the ion channel, as described above.<sup>88-90</sup> Similar scenarios have been reported for other class A GPCRs, as well as bioaminergic transporters and ion channels, which, all put together, represent a significant proportion of targets featured in the CNS discovery portfolios across the industry.

Over the years an increasing body of information has been accumulated in the literature on the medicinal chemistry strategy and tactics for overcoming blockade of the hERG channel. To leverage this wealth of publically available information a detailed pairwise analysis of the reported optimization efforts reported in the literature was carried out with the aim of producing a set of simple, empirical guidelines for attenuating hERG activity. This led to identification of four distinct strategies: control of logP, attenuation of pK<sub>a</sub>, Discrete Structural Modifications (DSM) and formation of zwitterions (ZI), as discussed below.<sup>87</sup>

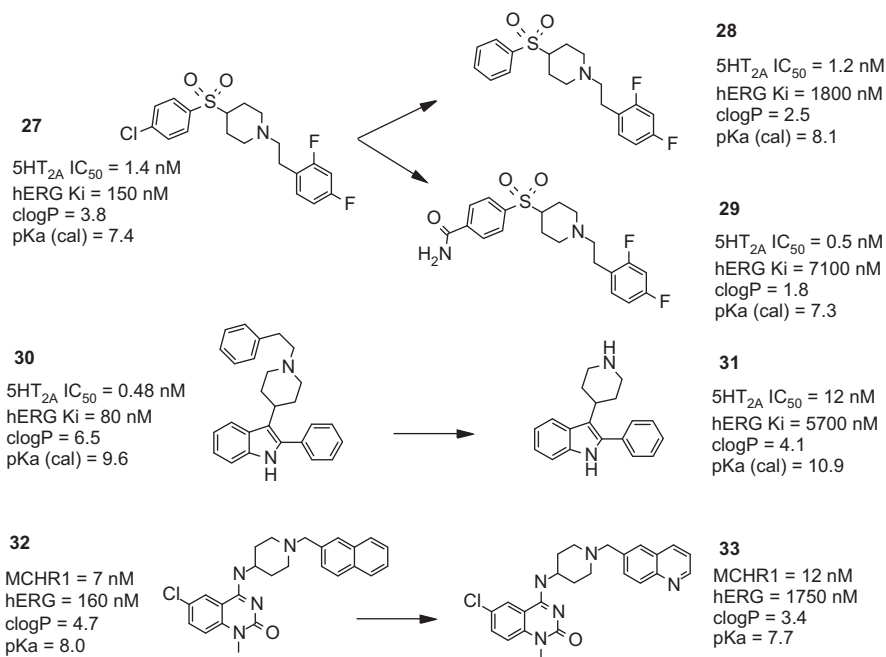
### 18.3.1.1 Control of clogP

SAR data can often show series-dependent correlation between potency of hERG binding and measures of lipophilicity.<sup>87,95</sup> This is supported by the hERG homology models and mutagenesis data,<sup>88,90</sup> suggesting the existence of a drug binding site within a large hydrophobic cavity aligned with aromatic residues. By reducing lipophilicity of a drug molecule, interaction with the hydrophobic cavity will be destabilized, resulting in decreased hERG activity.

For example, Fletcher and coworkers at Merck effectively employed the control of *clogP* strategy to improve selectivity over hERG in a series of 5-HT<sub>2A</sub> antagonists represented by lead compound **27** (*clogP* 3.8, hERG K<sub>i</sub> 150 nM). This was achieved by deletion of the Cl atom (**28**, *clogP* 2.5, hERG K<sub>i</sub> 1800 nM), or by its replacement with a polar amide group (**29**, *clogP* 1.8, hERG K<sub>i</sub> 7100 nM).<sup>96</sup> In both examples the primary 5-HT<sub>2A</sub> potency remained unaffected (Figure 18.7).

In another series of 5-HT<sub>2A</sub> antagonists reported by Merck Sharp and Dohme,<sup>97</sup> the authors hypothesized that the phenethyl group in the lead compound **30** confers high activity at hERG (K<sub>i</sub> = 80 nM). Indeed, deletion analogue **31** displayed significantly reduced hERG binding K<sub>i</sub> of 5.7  $\mu$ M consistent with its lower *clogP* (4.1), thus providing an attractive point for further optimization.

In fact, reduction of the number of aromatic rings is often a successful medicinal chemistry strategy when it comes to improving selectivity against not only hERG, but also CYPs, aqueous solubility, serum album binding and a number of other parameters that impact a compound's overall developability.<sup>98</sup> Indeed, the mean aromatic ring count was found to decline as compounds advance through clinical trials, suggesting that compounds with fewer aromatic rings are more likely to be successful in development. The average number of aromatic rings in FDA approved oral drugs is 1.6.<sup>99</sup> This is consistent with findings of a complementary study that the fraction of sp<sup>3</sup> hybridized carbon



**Figure 18.7** Examples of *clogP* control strategy.



atoms ( $F_{sp3}$  = Number of  $sp^3$  hybridized carbon atoms/total carbon atom count) increases with progression through the development process.<sup>100</sup> Of course, it is not always possible to delete an aromatic group without significantly affecting activity for the primary target. In such cases replacing the carboaromatic (*e.g.* phenyl rings and benzo-fused ring systems) with a corresponding heteroaromatic group may prove a more successful approach. It has been shown that the detrimental impact of increasing aromatic ring count is driven mainly by the carboaromatic ring component – for any given number of aromatic rings in a molecule the higher the carboaromatic with respect to heteroaromatic content the greater risk of failure in development. It is therefore advisable not only to limit the overall aromatic rings in a molecule but also, where possible, to replace carboaromatics with heteroaromatic congeners (whilst keeping an eye on properties required for CNS exposure, such as polar surface area and number of H-bond donors).<sup>101</sup>

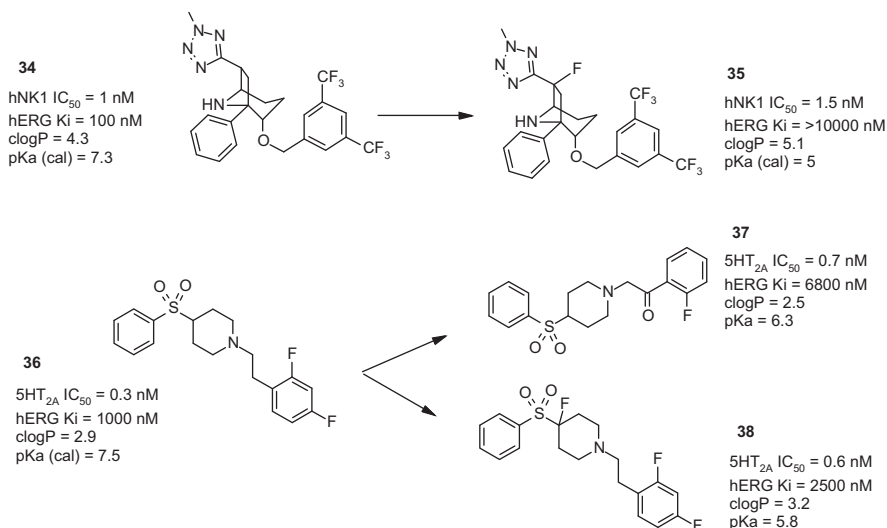
This was exactly the approach adopted by Blackburn *et al.*<sup>102</sup> in their efforts to improve hERG selectivity in their series of MCHR1 antagonists. Replacement of the naphthyl group of **32** with a quinolyl group produced **33** (Figure 18.7) with significantly reduced lipophilicity (1.3 log units) and improved selectivity over hERG from around 20- to almost 200-fold.

### 18.3.1.2 Attenuation of $pK_a$

Many of the ligands that block hERG contain a basic nitrogen moiety, which is likely to be protonated at physiological pH. However, it should be noted that a basic amine is certainly not a pre-requisite to hERG blockade, as a suitably placed aromatic or hydrophobic group in the ligand can potentially interact with the channel equally well. Based on mutagenesis and homology modelling, it has been proposed that  $\pi$ -cation interactions between basic amine containing hERG blockers and aromatic residues within the cavity of the hERG channel contribute to ligand binding affinity.<sup>88,89</sup> Therefore, lowering the  $pK_a$  of a basic nitrogen would reduce the proportion of molecules in the protonated form at physiological pH, and would be expected to disrupt any putative  $\pi$ -cation interactions with the channel. In general, it has been observed that modification of  $pK_a$  can often have the effect of increasing the polarity of a compound (*e.g.* piperidine to piperazine) and thus can have the same net impact as logP.

The  $pK_a$  lowering was adopted by Merck scientists in their efforts to improve hERG selectivity within a new series of conformationally constrained hNK1 antagonists.<sup>105</sup> The lead compound N-2 methyltetrazole analogue **34** (Figure 18.8) had an attractive profile with high hNK1 affinity (1 nM) and long lasting *in vivo* efficacy in the foot-tapping gerbil paradigm; however, it was compromised by a hERG liability ( $K_i$  0.1  $\mu$ M). Introduction of a fluorine at the C-6 position of the azabicyclic ring to give **35** (calc  $pK_a$  = 5.00) maintained the hNK1 affinity observed in the parent des-fluoro analogue **11** (calc.  $pK_a$  = 7.3), but dramatically increased the selectivity over hERG ( $K_i$  > 10  $\mu$ M).

Returning to the 5-HT<sub>2A</sub> area, Fletcher *et al.*<sup>96</sup> demonstrated how control of  $pK_a$  can be used to gain additional selectivity over hERG. Compound **36** was



**Figure 18.8** Examples of pK<sub>a</sub> attenuation strategy.

found to prolong the QT interval in the anaesthetized ferret by more than 10% at doses of 3 and 10 mg/kg/h. A range of modifications were made to **36** in order to further improve selectivity over hERG. Some success was achieved through introduction of a ketone at the  $\beta$ -position to the amine (**37**, Figure 18.8). Such attenuation of the pK<sub>a</sub> of the piperidine system yields a commensurate reduction in activity at hERG. The optimized compound did not show QT prolongation in the anaesthetized ferret model at doses up to 10 mg/kg/h and exhibited acceptable pharmacokinetic properties. Despite these improvements, compound **37** was found to degrade in polar solvents such as methanol due to the presence of the ketone moiety, and therefore could not be developed further.<sup>104</sup> As control of pK<sub>a</sub> was found to be of utility in governing activity at hERG, the group sought an additional approach to modulating pK<sub>a</sub>. In this case a series of 4-fluorosulfones was targeted. These were found to provide acceptable levels of selectivity over hERG, with compound **38** showing no increase in QT prolongation in anaesthetized dogs at plasma concentrations up to 148  $\mu$ M.

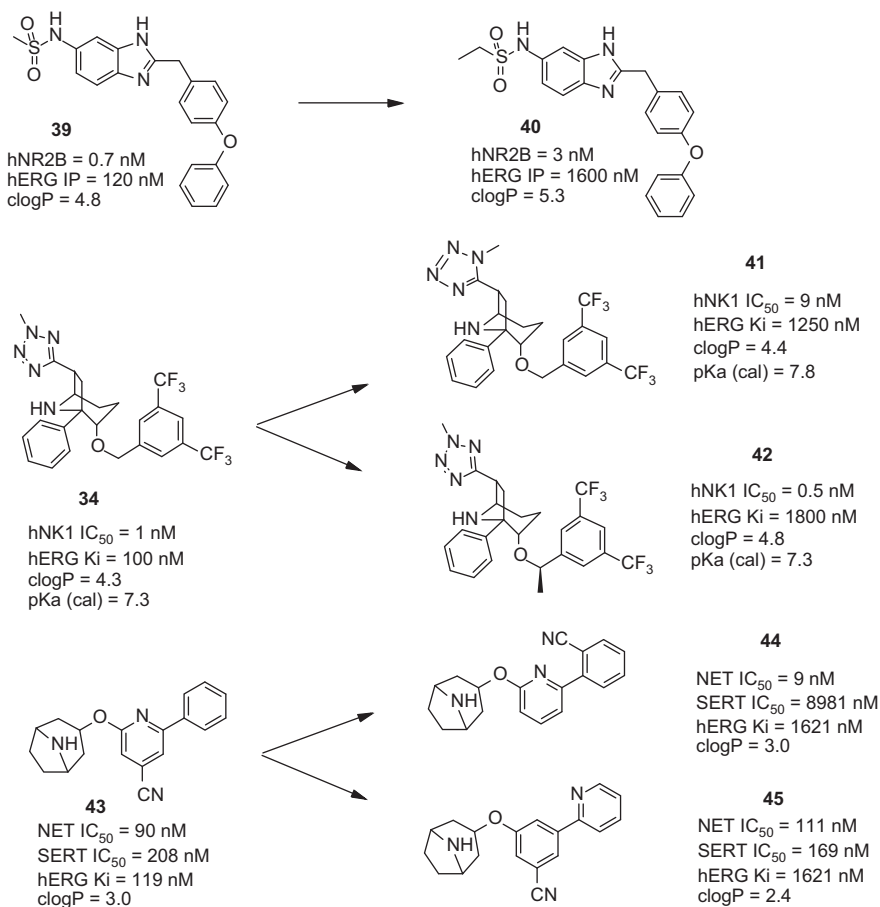
### 18.3.1.3 Discrete Structural Modifications (DSM)

Discrete, often peripheral, modifications to a drug molecule can have a dramatic effect on hERG potency, a phenomenon potentially explained by disruption of the putative interactions with aromatic residues (F656 and Y652) lining the hERG channel. Strategies adopted to mitigate hERG activity in this way are not restricted to modifications to distal aryl rings, but can also include introducing constraint and variation in stereochemistry. Criteria for including optimization pairs in this category are as follows: one clear site of modification, >1 log reduction in hERG activity, no significant reduction (<1 log unit) in

key physicochemical parameters such as  $\text{clogP}$  and  $\text{pK}_a$  and maintained activity at the primary target.

A particularly interesting example in this category is a benzimidazole series of NMDA NR2B antagonists reported by researchers at Merck.<sup>105</sup> Lead compound **39** (Figure 18.9), lacking a basic nitrogen that is often perceived as one of the key determinants of high hERG activity, displays high potency for the NR2B channel ( $K_i = 0.7 \text{ nM}$ ) but also shows high affinity for hERG in the MK-499 binding assay (IP  $120 \text{ nM}$ ). A simple change of the methyl sulfonamide of **39** to the ethyl sulfonamide in **40** resulted in a 13-fold decrease in hERG affinity. The reduction in hERG affinity may be attributed to specific structural changes to the periphery of the molecule potentially disrupting the interaction of the adjacent aromatic ring with the hERG channel binding site.

As discussed earlier, attenuation of  $\text{pK}_a$  as a strategy, initially pursued by Huscroft and his colleagues at Merck,<sup>103</sup> to improve hERG selectivity in their



**Figure 18.9** Examples of Discrete Structural Modifications.

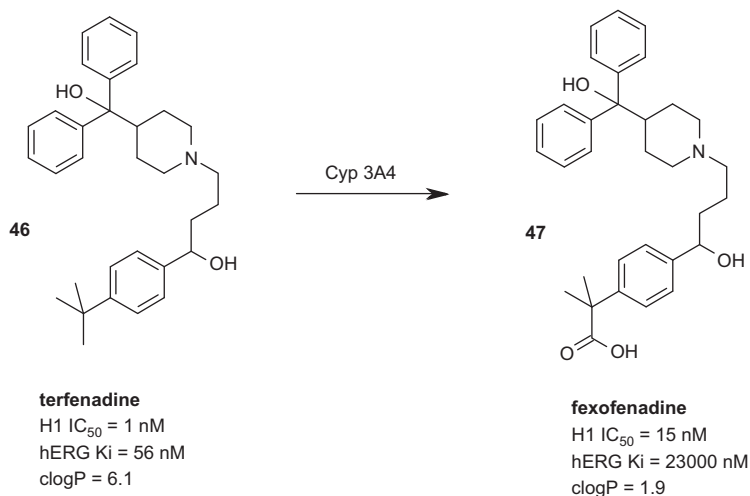
new series of conformationally constrained hNK1 antagonists was successful. Introduction of a fluorine in the azabicyclic ring of **34** resulted in **35** with significantly improved selectivity over hERG (Figure 18.8). However, this change was also accompanied by a reduction in duration of action *in vivo*. A closely related N – 1 methyl analogue **41** displayed ~12-fold lower hERG binding ( $K_i$  1.25  $\mu$ M), indicating high sensitivity of hERG SAR to subtle structural changes in this series (Figure 18.9). A more striking DSM example in this particular series derived from introduction of an  $\alpha$ -methyl substituent into the pendant benzyl ether side chain to give **42**, which resulted in attenuated hERG activity ( $K_i$  1.8  $\mu$ M) whilst maintaining hNK1 affinity (0.5 nM) and *in vivo* efficacy.<sup>103</sup>

Another interesting example of discrete hERG SAR was described by Bingham *et al.* in a report on optimization of a dual norepinephrine and serotonin transporter, NERT/SERT, inhibitors for the treatment of pain.<sup>106</sup> The lead compound **43** displayed desirable NET/SERT profile; however, it also proved to be an equipotent hERG blocker (119 nM). In their efforts to improve the hERG selectivity, authors found that shifting the nitrile group in **43** to the distal aromatic ring as in **44** resulted in reduction of the hERG potency by 60-fold. Unfortunately, although a potent NET inhibitor, this compound showed a very low potency at SERT, which highlights additional challenges faced by medicinal chemists involved in multi-target drug discovery.<sup>107</sup> Interestingly, shifting the nitrogen instead of the nitrile to the same position in the distal phenyl ring, as in **45**, led to significantly reduced hERG, whilst not effecting NET and SERT potency.<sup>106</sup>

#### 18.3.1.4 Incorporation of Carboxylic Group

As a potassium cation channel, hERG has evolved to stabilize positive charge within its central cavity, which may, at least in part, explain why many hERG blockers contain basic amine functionality that can be protonated under normal physiological conditions. This may also rationalize the fact that the presence of functionality that is negatively charged at physiological pH, such as a carboxylic group, is almost universally detrimental to hERG binding.<sup>108</sup>

This effect was for the first time observed with terfenadine (**46**, Figure 18.10), the progenitor in the second generation of antihistamines launched in 1982. Due to instances of cardiac arrhythmia, terfenadine was withdrawn from the market in 1997. However, it was subsequently discovered that its principal metabolite, carboxylate **47**, not only accounts for all of the therapeutic effect of terfenadine, but it also displays significantly reduced hERG affinity and no effect on QT interval.<sup>109</sup> This metabolite was subsequently marketed as fexofenadine, the first of the third generation of antihistamines, characterized by a lack of central side-effects intrinsic to the prior generations. The improved side-effect profile has been attributed to low CNS exposure of the zwitterionic fexofenadine. Since compounds containing carboxylic or zwitterionic groups tend to have poor CNS exposure,<sup>110</sup> this approach is limited mainly to peripheral targets.



**Figure 18.10** Terfenadine and its main metabolite, fexofenadine.

### 18.3.1.5 Medicinal Chemistry vs. hERG: Recommendations

Based on the literature data summarized here and in an earlier publication<sup>87</sup> we can propose the following recommendations for projects facing hERG selectivity challenge:

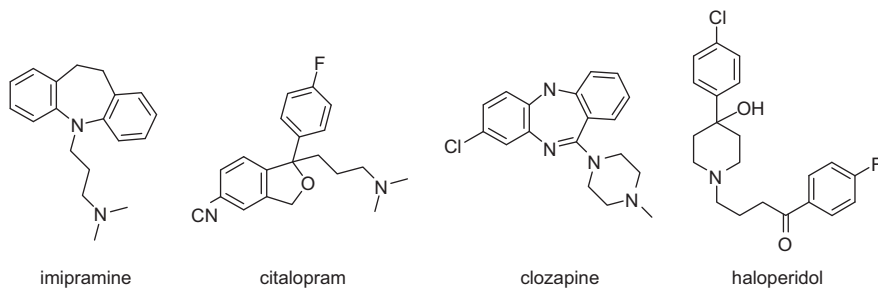
- Four strategies for the removal of hERG have been identified: Discrete Structural Modifications, control of logP, formation of zwitterions and control of pK<sub>a</sub>.
- All four strategies were found to be equally efficacious in diminishing hERG activity. Literature evidence indicates that the zwitterion approach is of lower general applicability owing to issues with membrane permeability, oral bioavailability and CNS exposure.
- Consideration of clogP of the starting compound enables selection of the most appropriate strategy for lowering hERG activity.
  - Where clogP ≥ 3.0: seek to reduce this by *e.g.* incorporation of heteroatoms, polar groups or removal of lipophilic moieties. On average 1 log unit reduction in clogP leads to 0.8 log unit reduction in hERG activity. If no correlation between hERG activity and logP can be established in the series then pursue the DSM strategy.
  - If clogP < 3.0: Discrete Structural Modifications offer the highest probability of success.
- A corollary to the above is to remove or modify aryl moieties in the target molecule. This may have the effect of reducing clogP and potentially disrupting  $\pi$ -stacking with the channel.
- Reducing pK<sub>a</sub> of a basic nitrogen is a frequently employed strategy in attenuating hERG. This is often associated with reduction in logP, which may be the more relevant parameter.

- A basic nitrogen (although not a prerequisite) is often associated with hERG activity; therefore, alternative solubilizing groups should be considered.
- hERG data should be interpreted in the context of measured solubility. Compounds with low aqueous solubility (<5 mg/l) may have significantly underestimated activity in the hERG assay.
- Only optically pure material should be tested in the hERG assay as the effects due to stereochemistry can be dramatic.
- To ensure the effects of discrete structural modifications are identified and to establish reliable SAR, test a significant number of analogues within each chemical series against hERG.
- Where the amount of data available permits, consider development of a local (series specific) *in silico* model to guide medicinal chemistry efforts away from hERG activity.
  - Application of global *in silico* models is best reserved for prioritization of compounds for synthesis/acquisition from larger arrays.
- To ascertain effects of non-hERG mediated QT prolongation, test key compounds in relevant *ex vivo* (e.g. dog purkinje fibres) or *in vivo* models as early as possible.

### 18.3.2 Phospholipidosis

Phospholipidosis is a storage disorder characterized by the excess accumulation of phospholipids in tissues. It can occur as a result of metabolic dysfunction or genetic disorder, or can arise following chronic exposure to cationic lipophilic drugs; so-called drug-induced phospholipidosis (DIPL).<sup>111–113</sup> In the latter case the increase in cellular phospholipids, and the subsequent morphological changes that can be seen by electron microscopy, such as the appearance of lamellated inclusion bodies and macrophage infiltration, are reversible following cessation of drug exposure. Although there is no conclusive evidence for a causal link between the phospholipidosis seen preclinically and toxicological outcomes in the clinic, indeed many marketed antidepressants and over 50 marketed and experimental drugs are known to cause DIPL.<sup>113,114</sup> The known association between Niemann–Pick type C disease and phospholipidosis has made regulatory bodies hesitant to approve drugs that induce DIPL. The visible morphological changes in tissues are cause for further concern. Historically the lack of suitable biomarkers for phospholipidosis has led pharmaceutical companies to screen out problem compounds during development. Whether the emergence of potential clinical biomarkers such as bis(monoacylglycerol)phosphate will change this stance remains to be seen.<sup>115</sup>

Drug-induced phospholipidosis is most commonly caused by cationic lipophilic drugs, often referred to as cationic amphiphilic drugs (CADs).<sup>116</sup> Since this represents a common pharmacophore for monoamine receptors it is not surprising that several antidepressants and antipsychotics are known DIPLs (Figure 18.11). Although the issue of phospholipidosis is not exclusive to CNS drugs, the frequent observation of the CAD pharmacophore in this class of



**Figure 18.11** Examples of CNS drugs known to induce phospholipidosis.<sup>116</sup>

**Table 18.13** Predictions for the potential for phospholipidosis based on physicochemical properties (adapted from Meanwell<sup>73</sup>).

	<i>Predict positive for phospholipidosis</i>	<i>Predict negative for phospholipidosis</i>
Ploeman model	$(\text{most basic } pK_a)^2 + (\text{cLogP})^2 \geq 90$	$(\text{most basic } pK_a)^2 + (\text{cLogP})^2 \leq 90$
Modified Ploeman model	where $pK_a \geq 8$ and $\text{cLogP} \geq 1$ $(\text{most basic } pK_a)^2 + (\text{cLogP})^2 \geq 50$	or $pK_a < 8$ and $\text{cLogP} < 1$ $(\text{most basic } pK_a)^2 + (\text{cLogP})^2 \leq 50$
Tomizawa model	where $pK_a \geq 6$ and $\text{cLogP} \geq 2$ For $\text{cLogP} > 1$ : $1 \geq \text{Net Charge at pH } 4 \leq 2$ For $\text{cLogP} > 2.75$ : Net Charge at $\text{pH } 4 = 1$	or $pK_a < 6$ and $\text{cLogP} < 2$ Net Charge at $\text{pH } 4 < 1$ or $\text{cLogP} < 1.61$ : Net Charge at $\text{pH } 4 = 1$
Hanumegowda model	For $\text{cLogP} \geq 2$ most basic $pK_a \times \text{cLogP} \times V_d \geq 180$	$\text{cLogP} < 2$ or most basic $pK_a \times \text{cLogP} \times V_d < 180$

drug compounds merits its mention in this chapter. Several groups have proposed physicochemical guidelines for drug-induced phospholipidosis,<sup>117–121</sup> which have been elegantly summarized in a review by Meanwell<sup>73</sup> (Table 18.13). These physicochemical guidelines can be useful tools in the design process when phospholipidosis has been identified experimentally as a possible risk factor.<sup>73</sup>

## 18.4 Summary

The design of therapeutic agents that can penetrate the blood-brain barrier and achieve the drug concentrations required for efficacious target receptor occupancy in the brain region of interest is a unique and major challenge for medicinal chemists working on CNS targets. Direct measurements of receptor occupancy and drug concentration in brain interstitial fluid are labour-intensive and low-throughput methods. Consequently, due to relatively simple and straightforward sampling, CSF concentration is widely used as a surrogate



marker for unbound drug in the brain to guide CNS optimization efforts. However, CSF can only serve as a good surrogate for drugs that are not P-gp substrates and have good membrane permeability. It is, therefore, important to use these three parameters (CSF, P-gp and permeability) together with plasma unbound fraction as key criteria to guide lead optimization efforts.

In addition, a good understanding of the fundamental relationships between physicochemical properties and *in vitro* and *in vivo* outcomes is now widely considered as the basic requirement for prospective design of compounds with overall favourable DMPK and safety profile. Lipophilicity ( $\text{clogP}/\text{clogD}$ ), molecular weight (MW), total polar surface area (TPSA), basicity ( $\text{pK}_a$ ), number of hydrogen bond donors (HBD) and number of rotatable bonds (RB) are considered the most critical molecular attributes, and their median value derived from analysis of marketed CNS drugs by Wager and colleagues at Pfizer defines an optimal and desirable CNS candidate profile (Table 18.14).<sup>27</sup>

In addition to potency and DMPK profile, physicochemical properties also impact drug safety. Presence of a basic amine and level of lipophilicity warranting good BBB permeability are common features amongst CNS active agents. However, these have also been directly associated with a range of safety-related issues, including hERG block and phospholipidosis. Keeping  $\text{clogP}$  and  $\text{pK}_a$  low (*e.g.* below 3 and 9, respectively) is an effective strategy to minimize safety risks.

Binding efficiency concepts introduced at the turn of the century are now widely utilized by medicinal chemists striving to maximize the ligand potency whilst still keeping physicochemical properties within an optimal range consistent with good DMPK and safety profile. Ligand efficiency ( $\text{LE} = \Delta G_{\text{bind}}/\text{number of heavy atoms}$ ) was the first of the efficiency indices introduced by Hopkins in 2004 to express efficiency of ligand binding (Gibbs free energy) in respect to its size, as measured by the number of heavy atoms.<sup>50</sup> Recognizing the importance of lipophilicity as one of the most critical molecular attributes, ligand-lipophilicity efficiency has also been proposed ( $\text{LLE} = \text{pK}_i$ ,  $\text{pK}_d$  or  $\text{pIC}_{50} - \text{clogP}$ ).<sup>51</sup> More recently Keseru and Makara suggested ligand-efficiency-dependent lipophilicity, which factors in both size and lipophilicity ( $\text{LELP} = \text{clogP}/\text{LE}$ ).<sup>122</sup> These efficiency parameters can help medicinal chemists to take a more holistic approach when selecting and optimizing leads, and ultimately deliver higher quality clinical candidates. The median values reported by Wager *et al.* in his elegant property analysis of the CNS drugs for LE, LLE and LELP (0.52 and 6.3 and 5.9, respectively) provide a useful guidance for CNS drug discovery.<sup>27</sup>

As discussed above, the modern medicinal chemist working in CNS discovery monitors an ever-increasing set of calculated and experimental data.

**Table 18.14** Physicochemical properties of marked CNS drugs (median values).

$\text{clogP}$	$\text{clogD}$	$\text{MW (D)}$	$\text{TPSA (Å}^2\text{)}$	$\text{pK}_a$	HBD	$\text{RB}^{23}$
2.8	1.7	305.3	44.8	8.4	1	4.5

The ability to process these data effectively and engage in multi-parameter prospective design ultimately determines the success in delivering high-quality drug candidates that are suitable to test robustly hypotheses in the clinic and have good probability of reaching the market.

Thus, the current state-of-the-art in CNS therapeutics remains limited by the types of molecules that can be delivered to the brain. Progress in DMPK and pharmacology has led to increased access to surrogate models of brain exposure such as serial CSF; however, determining receptor occupancy remains a resource intensive process and technical advances in this area to improve throughput will greatly facilitate CNS medicinal chemistry. While the strategies outlined above will enable improved design of small-molecule drugs, a deeper understanding of transport mechanisms at the BBB is required before broader scope in terms of CNS-active chemical structures can be envisaged. For example, several areas of future potential interest in CNS therapy, such as siRNA and protein–protein interactions, may require new CNS delivery paradigms which will all likely require non-“rule-of-five” molecules.

## References

1. N. J. Abbott, L. Rönnbäck and E. Hansson, *Nat. Rev. Neurosci.*, 2006, **7**, 41.
2. W. M. Pardridge, *NeuroRx: J. Am. Soc. Exp. NeuroTherapeutics*, 2005, **2**, 3.
3. D. D. Shen, A. A. Artru and K. K. Adkison, *Adv. Drug Delivery Rev.*, 2004, **56**, 1825.
4. T. Kageyama, M. Nakamura, A. Matsuo, Y. Yamasaki, Y. Takakura, M. Hashida, Y. Kanai, M. Naito, T. Tsuruo, N. Minato and S. Shimohama, *Brain Res.*, 2000, **879**, 115.
5. A. King, *Chem. World*, 2011, **8**(6), 36.
6. W. M. Pardridge, *Drug Discovery Today*, 2007, **12**(1/2), 54.
7. I. Martin, *Drug Discovery Today*, 2004, **9**(4), 161.
8. W. M. Pardridge, *Drug Discovery Today*, 2004, **9**(9), 392.
9. X. Liu and C. Chen, *Curr. Opin. Drug Discovery Dev.*, 2005, **8**, 506.
10. X. Liu, B. J. Smith, C. Chen, E. Callegari, S. L. Becker, X. Chen, J. Cianfrogna, A. C. Doran, S. D. Doran, J. P. Gibbs, N. Hosea, J. Liu, F. Nelson, M. A. Szezw and J. V. Deussen, *J. Pharmacol. Exp. Ther.*, 2005, **313**, 1254.
11. T. S. Maurer, D. B. DeBartolo, D. A. Tess and D. O. Scott, *Drug Metab. Dispos.*, 2005, **33**, 175.
12. S. G. Summerfield, A. J. Stevens, L. Cutler, O. M. del Carmen, B. Hammond, S.-P. Tang, A. Hersey, D. J. Spalding and P. Jeffrey, *J. Pharmacol. Exp. Ther.*, 2006, **316**, 1282.
13. A. Reichel, *Chem. Biodiversity*, 2009, **9**, 2030.
14. K. M. Mahar Doan, J. E. Humphreys, L. O. Webster, S. A. Wring, L. J. Shampine, C. J. Serabjit-Singh, Kimberly K. Adkinson and J. W. Polli, *J. Pharmacol. Exp. Ther.*, 2002, **303**, 1029.

15. D. F. Veber, S. R. Johnson, H.-Y. Chen, B. R. Smith, K. W. Ward and K. D. Kopple, *J. Med. Chem.*, 2002, **45**, 2615.
16. E. R. Zhang, M. A. Phelps, C. Cheng, S. Elkins and P. W. Swaan, *Adv. Drug Deliv. Rev.*, 2002, **54**, 329 and references therein.
17. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Del. Rev.*, 1997, **23**, 3–25.
18. H. Van de Waterbeemd and M. Kansy, *Chimia*, 1992, **46**, 299.
19. J. A. Gratton, M. H. Abraham, M. W. Bradbury and H. S. Chadra, *J. Pharm. Pharmacol.*, 1997, **49**, 1211.
20. H. Van de Waterbeemd, G. Camenisch, G. Folkers, J. R. Chretien and O. A. Raevsky, *J. Drug Targeting*, 1998, **6**, 151.
21. U. Norinder and M. Haeberlein, *Adv. Drug Deliv. Reviews*, 2002, **54**, 291.
22. R. Didziapetris, P. Japertas, A. Avdeef and A. Petrauskas, *J. Drug Targeting*, 2003, **11**, 391.
23. P. D. Leeson and A. M. Davis, *J. Med. Chem.*, 2004, **47**, 6338.
24. H. Pajouhesh and G. R. Lenz, *NeuroRx: J. Am. Chem. Soc. Exp. Neuro-Therapeutics*, 2005, **2**, 541.
25. S. A. Hitchcock and L. D. Pennington, *J. Med. Chem.*, 2006, **49**, 7559.
26. M. P. Gleeson, *J. Med. Chem.*, 2008, **51**, 817.
27. M. J. Waring, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 2844.
28. T. T. Wager, R. Y. Chandrasekaran, X. Hou, M. D. Troutman, P. R. Verhoest, A. Villalobos and Y. Will, *ACS Chem. Neurosci.*, 2010, **1**, 420.
29. T. T. Wager, X. Hou, P. R. Verhoest and A. Villalobos, *ACS Chem. Neurosci.*, 2010, **1**, 435.
30. S. P. Gupta, *Chem. Rev.*, 1989, **89**, 1765 and references therein.
31. C. A. S. Bergström, M. Strafford, L. Lazorova, A. Avdeef, K. Luthman and P. Artursson, *J. Med. Chem.*, 2003, **46**, 558.
32. V. Tantishaiyakul, *Pharmazie*, 2001, **56**, 407.
33. Y. C. Martin, *J. Med. Chem.*, 2005, **48**, 3164.
34. W. J. Egan, K. M. Merz, Jr and J. J. Baldwin, *J. Med. Chem.*, 2000, **43**, 3867.
35. Q. R. Smith and Y. Takasato, *Ann. NY Acad. Sci.*, 1986, **481**, 186.
36. J. A. Gratton, M. H. Abraham, M. W. Bradbury and H. S. Chada, *J. Pharm. Pharmacol.*, 1997, **49**, 1211.
37. H. Murakami, H. Takanaga, H. Matsuo, H. Ohtani and Y. Sawada, *Am. J. Physiol.*, 2000, **279**, H1022.
38. X. Liu, M. Tu, R. S. Kelly, C. Chen and B. J. Smith, *Drug Metab. Dispos.*, 2004, **32**, 132.
39. D. S. Middleton, M. Andrews, P. Glossop, G. Gymer, A. Jessiman, P. S. Johnson, M. MacKenny, M. J. Pitcher, T. Rooker, A. Stobie, K. Tang and P. Morgan, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 1434.
40. D. J. Johnson, I. T. Forbes, S. P. Watson, V. Garzya, G. I. Stevenson, G. R. Walker, H. S. Mudhar, S. J. Flynn, P. A. Wyman, P. W. Smith, G. S. Murkitt, A. J. Lucas, C. R. Mookherjee, J. M. Watson, J. E. Gartlon, A. M. Bradford and F. Brown, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 5434.

41. P. W. Smith, P. A. Wyman, P. Lovell, C. Goodacre, H. T. Serafinowska, A. Vong, F. Harrington, S. Flynn, D. M. Bradley, R. Porter, S. Coggon, G. Murkitt, K. Searle, D. R. Thomas, J. M. Watson, W. Martin, Z. Wu and L. A. Dawson, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 837.
42. D. A. Smith, L. Di and E. H. Kerns, *Nat. Rev. Drug Discovery*, 2010, **9**, 929.
43. T. Österberg and U. Norinder, *J. Chem. Inf. Comput. Sci.*, 2000, **40**, 1408.
44. M. A. Abraham, *Eur. J. Med. Chem.*, 2004, **39**, 235.
45. M. Ahmed, M. A. Briggs, S. M. Bromidge, T. Buck, L. Campbell, N. J. Deeks, A. Garner, L. Gordon, D. W. Hamprecht, V. Holland, C. N. Johnson, A. D. Medhurst, D. J. Mitchell, S. F. Moss, J. Powles, J. T. Seal, T. O. Stean, G. Stemp, M. Thompson, B. Trail, N. Upton, K. Winborn and D. R. Witty, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4867.
46. J. Kelder, P. D. J. Grootenhuys, D. M. Bayada, L. P. C. Delbressine and J.-P. Ploemen, *Pharmaceutical Res.*, 1999, **16**, 1514.
47. D. E. Clark, *J. Pharmaceutical Sci.*, 1999, **88**, 807.
48. E. Pinnard, A. Alanine, D. Alberati, M. Bender, E. Borroni, P. Bourdeaux, V. Brom, S. Burner, H. Fischer, D. Hainzl, R. Halm, N. Hauser, S. Jolidon, J. Lengyel, H.-P. Marty, T. Meyer, J.-L. Moreau, R. Mory, R. Narquizian, M. Nettekoven, R. D. Norcross, B. Puellmann, P. Schmid, S. Schmitt, H. Stalder, R. Wermuth, J. G. Wettstein and D. Zimmerli, *J. Med. Chem.*, 2010, **53**, 4603.
49. S. Shengguo and A. Adejare, *Curr. Top. Med. Chem.*, 2006, **14**, 1457.
50. V. A. Levin, *J. Med. Chem.*, 1980, **23**, 682.
51. A. L. Hopkins, C. R. Groom and A. Alex, *Drug Discovery Today*, 2004, **9**, 430.
52. P. D. Leeson and B. Springthorpe, *Nat. Rev. Drug Discovery*, 2007, **6**, 881.
53. P. R. Verhoest, A. S. Basak, V. Parikh, M. Hayward, G. W. Kauffman, V. Paradis, S. F. McHardy, S. McLean, S. Grimwood, A. W. Schmidt, M. Vanase-Frawley, J. Freeman, J. Van Deusen, L. Cox, D. Wong and S. Liras, *J. Med. Chem.*, 2011, **54**, 5868.
54. J.-U. Peters, T. Lübbers, A. Alanine, S. Kolczewski, F. Blasco and L. Steward, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 262.
55. S. M. Bromidge, A. M. Brown, S. E. Clarke, K. Dodgson, T. Gager, H. L. Grassam, P. M. Jeffrey, G. F. Joiner, F. D. King, D. N. Middlemiss, S. F. Moss, H. Newman, G. Riley, C. Routledge and P. Wyman, *J. Med. Chem.*, 1999, **42**, 202.
56. G. Trani, S. M. Baddeley, M. A. Briggs, T. T. Chuang, N. J. Deeks, C. N. Johnson, A. A. Khazragi, T. L. Mead, A. D. Medhurst, P. H. Milner, L. P. Quinn, A. M. Ray, D. A. Rivers, T. O. Stean, G. Stemp, B. K. Trail and D. R. Witty, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5698.
57. M. Cowart, J. K. Pratt, A. O. Stewart, Y. L. Bannani, T. E. Esbenshade and A. A. Hancock, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 689.
58. V. A. Ashwood, M. J. Field, D. C. Horwell, C. Julien-Larose, R. A. Lewthwaite, S. McCleary, M. C. Pritchard, J. Raphy and L. Singh, *J. Med. Chem.*, 2001, **44**, 2276.

59. E. H. Demont, S. Arpino, R. A. Bit, C. A. Campbell, N. Deeks, S. Desai and S. J. Dowell, P. Gaskin, J. R. J. Gray, L. A. Harrison, A. Haynes, T. D. Heightman, D. S. Holmes, P. G. Humphreys, U. Kumar, M. A. Morse, G. J. Osborne, T. Panchal, K. L. Philpott, S. Taylor, R. Watson, R. Willis and J. Witherington, *J. Med. Chem.*, 2011, **54**, 6724.
60. A. Seelig, *Eur. J. Biochem.*, 1998, **251**, 252.
61. V. K. Gombar, J. W. Polli, J. E. Humphreys, S. A. Wring and C. S. Serabjit-Singh, *J. Pharm. Sci.*, 2004, **93**, 957.
62. E. Y. Zhang, M. A. Phelps, C. Cheng, S. Elkins and P. W. Swaan, *Adv. Drug Deliv. Rev.*, 2002, **54**, 329.
63. F. Broccatelli, E. Carosati, A. Neri, M. Frosini, L. Goracci, T. I. Oprea and G. Cruciani, *J. Med. Chem.*, 2011, **54**, 1740.
64. A. W. Ravna, G. Sager, S. G. Dahl and I. Sylte, in *Transporters as Targets for Drugs*, ed. M. Bingham and S. Napier, Springer, 2009, p. 15.
65. K. M. Giacomini, S.-M. Huang, D. J. Tweedie, L. Z. Benet, K. L.R. Brouwer, X. Chu, A. Dahlin, R. Evers, V. Fischer, K. M. Hillgren, K. A. Hoffmaster, T. Ishikawa, D. Keppler, R. B. Kim, C. A. Lee, M. Niemi, J. W. Polli, Y. Sugiyama, P. W. Swaan, J. A. Ware, S. H. Wright, S. W. Yee, M. J. Zamek-Gliszczynski and L. Zhang, *Nat. Rev. Drug Discovery*, 2010, **9**, 215.
66. K. Linnetemil and T. B. Ejsing, *Eur. Neuropsychopharmacol.*, 2008, **18**, 157.
67. F. Wakenhut, G. A. Allan, P. V. Fish, M. J. Fray, A. C. Harrison, R. McCoy, S. C. Phillips, T. Ryckmans, A. Stobie, D. Westbrook, S. L. Westbrook and G. A. Whitlock, *Bioorg. Med. Chem. Lett.*, 2001, **19**, 5078.
68. P. V. Fish, A. Harrison, F. Wakenhut and G. A. Whitlock, in *Accounts in Drug Discovery: Case Studies in Medicinal Chemistry*, ed. J. C. Barrish, P. H. Carter, P. T. W. Cheng and R. Zahler, Royal Society of Chemistry, 2011, p. 267.
69. K. Lu, Y. Jiang, B. Chen, E. M. Eldemenky, G. Ma, M. Packiarajan, G. Chandrasena, A. D. White, K. A. Jones, B. Li and S.-P. Hong, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 5310.
70. A. Vasudevan, A. J. Souers, J. C. Freeman, M. K. Verzal, J. Gao, M. M. Mulhern, D. Wodka, J. K. Lynch, K. M. Engstrom, S. H. Wagaw and S. Brodjian, B. Dayton, D. H. Falls, E. Bush, M. Brune, R. D. Shapiro, K. C. Marsh, L. E. Hernandez, C. A. Collins and P. R. Kym, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5293.
71. I. Kola, *Clin. Pharmacol. Ther.*, 2008, **83**, 227–230.
72. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeny, *Adv. Drug. Del. Rev.*, 1997, **23**, 3.
73. N. A. Meanwell, *Chem. Res. Toxicol.*, 2011, **24**, 1420.
74. M. S. Tute, *Adv. Drug Res.*, 1971, **6**, 1–77.
75. H. Kubinyi, *Arzn. Forsch.*, 1979, **29**, 1067.
76. M. T. D. Cronin, *Curr. Comp. Aided Drug Des.*, 2006, **2**, 405.
77. J. D. Hughes, J. Blagg, D. A. Price, S. Bailey, G. A. DeCrescenzo, R. V. D. E. Ellsworth, Y. M. Fobian, M. E. Gibbs, R. W. Gilles, N. Greene, E. H. T. Krieger-Burke, J. Loesel, T. Wager, L. Whiteley and Y. Zhang, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4872.

78. R. J. Vaz and T. Klabunde, *Antitargets*, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2008.
79. R. B. Rothman, M. H. Baumann, J. E. Savage, L. Rauster, A. McBride, S. J. Hufeisen and B. L. Roth, *Circulation*, 2000, **102**, 2836.
80. J. H. Kehne, B. M. Carr, S. F. Chaney, J. Elands, D. J. Feldman, R. A. Frank, P. L. van Giersbergen, T. C. McCloskey, M. P. Johnson, D. R. McCarty, M. Poirot, Y. Senyah, B. W. Siegel and C. Widmaier, *J. Pharmacol. Exp. Ther.*, 1996, **277**, 968.
81. S. J. Peroutka, D. C. U'Prichard, D. A. Greenberg and S. H. Snyder, *Neuropharmacol.*, 1977, **16**, 549.
82. N. R. Mirza, D. Peters and R. G. Sparks, *CNS Drug Rev.*, 2003, **9**, 159.
83. M. Eichelbaum, N. Spannbrucker and H. J. Dengler, *Eu. J. Clin. Pharmacol.*, 1979, **16**, 189.
84. B. Fermini and A. A. Fossa, *Nat. Rev. Drug Discovery*, 2003, **2**, 439–447.
85. G. W. Abbott, F. Sesti, I. Splawski, M. E. Buck, M. H. Lehmann, K. W. Timothy, M. T. Keating and S. A. Goldstein, *Cell*, 1999, **97**, 175.
86. M. T. Keating and M. C. Sanguinetti, *Cell*, 2001, **104**, 569.
87. C. Jamieson, E. M. Moir, Z. Rankovic and G. Wishart, *J. Med. Chem.*, 2006, **49**, 5029.
88. J. S. Mitcheson, J. Chen, M. Lin, C. Culberson and M. C. Sanguinetti, *Proc. Natl Acad. Sci. USA*, 2000, **97**, 12329.
89. R. A. Pearlstein, R. J. Vaz, J. Kang, X.-L. Chen, M. Preobrazhenskaya, A. E. Shchekotikhin, A. M. Korolev, L. N. Lysenkova, O. V. Miroshnikova, J. Hendrix and D. Rampe, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 1829.
90. D. Fernandez, A. Ghanta, G. W. Kauffman and M. C. Sanguinetti, *J. Biol. Chem.*, 2004, **279**, 10120.
91. J. S. Mitcheson and M. D. Perry, *Curr. Opin. Drug Discov. Dev.*, 2003, **6**, 667.
92. J. L. Mendez-Andino and J. A. Wos, *Drug Discovery Today*, 2007, **12**, 972.
93. A. Johansson, *Expert Opin. Ther. Patents*, 2011, **21**, 905.
94. T. Ulven, T. M. Frimurer, J.-M. Receveur, P. B. Little, O. Rist, P. K. Nørregaard and T. Högberg, *J. Med. Chem.*, 2005, **48**, 5684.
95. M. J. Waring and C. Johnston, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1759.
96. S. R. Fletcher, F. Burkamp, P. Blurton, S. F. K. Cheng, R. Clarkson, D. O'Connor, D. Spinks, M. Tudge, M. B. van Niel, S. Patel, K. Chapman, R. Marwood, S. Sheppard, G. Bentley, G. P. Cook, L. J. Bristow, J. L. Castro, P. H. Hutson and A. M. MacLeod, *J. Med. Chem.*, 2002, **45**, 492.
97. M. Rowley, D. J. Hallet, S. Goodacre, C. Moyes, J. Cawforth, T. J. Sparey, S. Patel, R. Marwood, S. Thomas, L. Hitzel, D. O'Connor, N. Szeto, J. L. Castro, P. H. Hutson and A. M. MacLeod, *J. Med. Chem.*, 2001, **44**, 1603.
98. T. R. Ritchie and S. J. F. Macdonald, *Drug Discovery Today*, 2009, **14**, 1011.
99. M. M. Hann, A. R. Leach and Gavin Harper, *J. Chem. Inf. Comput. Sci.*, 2001, **41**, 865.



100. F. Lovering, J. Bikker and C. Humblet, *J. Med. Chem.*, 2009, **52**, 6752.
101. T. J. Ritchie, S. J. F. Macdonald, R. J. Young and S. Picket, *Drug Discovery Today*, 2011, **16**, 164.
102. C. Blackburn, M. J. LaMarche, J. Brown, J. Lee Che, C. A. Cullis, S. Lai, M. Maguire, T. Marsilje, B. Geddes, E. Govek, V. Kadambi and C. Doherty, B. Dayton, S. Brodjian, K. C. Marsh, C. A. Collins and P. R. Kym, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2621.
103. I. T. Huscroft, E. J. Carlson, G. G. Chicchi, M. M. Kurtz, C. London, P. Raubo, A. Wheeldon and J. J. Kulagowski, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 2008.
104. L. R. Fish, M. T. Gilligan, A. C. Humphries, M. Ivarsson, T. Ladduwahetty, K. J. Merchant, D. O'Connor, S. Patel, E. Philipps, H. M. Vargas, P. H. Hutson and A. M. MacLeod, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 3665–3669.
105. J. A. McCauley, C. R. Theberge, J. J. Romano, S. B. Billings, K. D. Anderson, D. A. Claremon, R. M. Freidinger, R. A. Bednar, S. D. Mosser, S. L. Gaul, T. M. Connolly, C. L. Condra, M. Xia, M. E. Cunningham, B. Bednar, G. L. Stump, J. J. Lynch, A. Macaulay, K. A. Wafford, K. S. Koblan and N. J. Liverton, *J. Med. Chem.*, 2004, **47**, 2089.
106. D. Angus, M. Bingham, D. Buchanan, N. Dunbar, L. Gibson, R. Goodwin, A. Haunso, A. Houghton, M. Huggett, R. Morphy, S. Napier, O. Nimz, J. Passmore and G. Walker, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 271.
107. Z. Rankovic and R. Morphy, *Curr. Pharmaceut. Design*, 2009, **15**, 587.
108. B.-Y. Zgu, Z. J. Jia, P. Zhang, T. Su, W. Huang, E. Goldman, D. Tumas, V. Kadambi, P. Eddy, U. Sinha, R. M. Scarborough and Y. Song, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 5507.
109. D. Rampe, B. Wibble, A. M. Brown and R. C. Dage, *Mol. Pharmacol.*, 1993, **44**, 1240.
110. C. G. Thomson, E. Carlson, G. G. Chicchi, J. J. Kulagowski, M. M. Kurtz, C. J. Swain, K. C. Tsao and A. Wheeldon, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 811–814.
111. M. J. Reasor and S. Kacew, *Exp. Biol. Med.*, 2001, **226**, 825.
112. U. P. Kodavanti and H. M. Mehendale, *Pharm. Rev.*, 1990, **42**, 327.
113. N. Anderson and J. Borlak, *FEBS Lett.*, 2006, **580**, 5533.
114. Z. Xia, G. Ying, A. L. Hansson, H. Karlsson, Y. Xie, A. Bergstrand, J. W. DePierre and L. Nässberger, *Prog. Neurobiol.*, 2000, **60**, 501.
115. E. A. Tengstrand, G. T. Miwa and F. Y. Hsieh, *Expert Opin. Drug Metab. Toxicol.*, 2010, **6**, 555.
116. H. Fischer, E.-A. Atzpodien, M. Csato, L. Doessegger, B. Lenz, G. Schmitt and T. Singer, *J. Med. Chem.*, 2012, **55**, 126.
117. J.-P. H. T. M. Ploemen, J. Kelder, T. Hafmans, H. van de Sandt, J. A. van Burgsteden, P. J. Salemink and E. van Esch, *Exp. Toxicol. Pathol.*, 2004, **55**, 347.
118. D. J. Pelletier, D. Gehlhaar, A. Tilloy-Ellul, T. O. Johnson and N. Greene, *J. Chem. Inf. Model*, 2007, **47**, 1196.



119. K. Tomizawa, K. Sugano, H. Yamada and I. Horii, *J. Toxicol. Sci.*, 2006, **31**, 315.
120. U. M. Hanumegowda, G. Wenke, A. Regueiro-Ren, R. Yordanova, J. P. Corradi and S. P. Adams, *Res. Toxicol.*, 2010, **23**, 749.
121. A. J. Ratcliffe, *Curr. Med. Chem.*, 2009, **16**, 2816.
122. G. M. Keserue and G. M. Makara, *Nat. Rev. Drug Discovery*, 2009, **8**, 203.

## CHAPTER 19

# *Multi-target Drug Discovery for Psychiatric Disorders*

ZORAN RANKOVIC\*<sup>a</sup> AND RICHARD MORPHY<sup>b</sup>

<sup>a</sup> Eli Lilly and Company, 893 South Delaware Street, Indianapolis, IN 46285, USA; <sup>b</sup> Eli Lilly and Company, Erl Wood Manor, Windlesham, Surrey, GU20 6PH, UK

\*Email: rankovic\_zoran@lilly.com

## 19.1 Introduction

The basic premise of the reductionist “one-target-one-disease” paradigm that has been dominating drug discovery over the past two decades is that selective modulation of a single protein would deliver a desired therapeutic effect with a good safety profile. Many successful drugs derived from this approach; however, emergence of this paradigm has notably coincided with a steady decline over the past two decades in the number of newly developed safe and effective drugs delivered to the market. Although research output over recent years has been trending in the right direction,<sup>1</sup> the overall performance of the pharmaceutical industry is still well below expectations fuelled by rocketing investments in R&D. It is estimated that only one out of nine clinical candidates makes it through development and receives approval by regulatory authorities.<sup>2</sup> This unsustainable attrition is attributed primarily to lack of efficacy, which may be due at least in part to the intrinsic redundancy and robustness of biological networks and a consequent failure of drugs modulating a single target to produce the desired therapeutic effect. Modulation of multiple targets within

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

relevant biological pathways and networks is increasingly recognized as a superior approach towards the next generation of treatments for diseases with complex, polygenic aetiology such as psychiatric disorders.<sup>3</sup> Consequently, there is an ever-increasing interest in developing agents that modulate specific multiple targets simultaneously (polypharmacology).<sup>4,5</sup>

Historically clinicians have treated unresponsive patients with a cocktail of drugs targeting different therapeutic mechanisms.<sup>6</sup> A major advantage of drug cocktails is the clinician's ability to titrate the dose, depending on the needs of an individual patient, to achieve optimal inhibition of each target. Since the benefits of this approach are often compromised by poor patient compliance, there has been a shift over the past decade or so towards fixed dose combination (FDC) drugs, whereby two, or more, drugs are co-formulated as a single tablet to make the dosing regimen simpler to follow. An alternative strategy is to develop a single chemical entity that is able to modulate multiple targets simultaneously. Many marketed drugs bind to more than one therapeutic target, but in most cases the multiple activity profile was serendipitous rather than designed.<sup>7</sup> A more recent trend in drug discovery has been a deliberate and rational design of ligands that act selectively on specific multiple targets (designed polypharmacology). These compounds have been described as Designed Multiple Ligands (DMLs), to distinguish them from the historical multi-target agents that often have poor selectivity and off-target effects. The process in which DMLs are discovered and optimized is referred to as Multi Target Drug Discovery (MTDD).<sup>8</sup>

## 19.2 Advantages and Disadvantages of FDCs and DMLs

The FDC approach is increasingly seen across the pharmaceutical industry not only as a solution to the compliance issues with drug cocktails, but also as an attractive life cycle management opportunity for successful drugs approaching the end of their patent coverage. Several FDCs are very successful commercially; however, there can be significant risks involved in the development of FDCs. Commercially, there is a good deal of uncertainty arising from the risk that clinicians might still prefer prescribing combinations of existing monotherapies that may offer greater dose flexibility and lower cost treatment in the case of generic drugs. One particularly important limitation of FDCs is that concomitant administration of two or more agents may result in drug-drug interactions and unacceptable additive toxicities. Furthermore, differences in the relative rates of metabolism between patients can produce highly complex pharmacokinetic (PK)/pharmacodynamic (PD) relationships for FDCs, leading to unpredictable variability between patients and necessitating extensive and expensive clinical studies. Difficulties of formulating multi-component capsules for acceptable stability over extended periods of time should not be underestimated and can be very costly and time-consuming. For example, the

components may interact with each other or may require different conditions for stability, such as a different pH.<sup>9</sup>

Compared to FDCs, the multiple ligand approach has a fundamentally different risk-benefit profile (Table 19.1). One of the downsides is increased complexity in the design and optimization of such ligands. However, this is a risk associated with an earlier and therefore less expensive stage of the drug discovery process. The risks and costs of developing DMLs are in principle no different from the development of any other single entity. Additional advantages of DMLs are lower risks of drug–drug interactions and a simplified PK/PD relationship relative to drug cocktails or FDCs.

It is important to recognize that drug cocktail, FDC and DML approaches have their own specific benefits and shortfalls and they all have their place in modern drug discovery. In fact, they should not be seen as mutually exclusive but potentially complementary approaches that in combination may offer an optimal therapeutic strategy, in particular when considering modulation of three or more targets and/or mechanisms.<sup>10</sup>

**Table 19.1** Risk-benefit profile of fixed dose combinations (FDCs) and designed multiple ligands (DMLs).

<i>Risks/benefits</i>	<i>Fixed dose combinations (FDCs)</i>	<i>Designed multiple ligands (DMLs)</i>
Patient compliance	Improved when compared to drug cocktails	Improved when compared to drug cocktails
PK/PD relationship	Often highly complex PK/PD correlation that requires sophisticated formulation solutions	Single chemical entity – generally no issues
Drug–drug interactions	Increased risk of drug–drug interactions	Risk similar to any other single compound entity
Titration of activities	Possible, but may be difficult and costly to develop – requires full clinical development, production and marketing of a series of dose combinations	Not possible
R&D challenges	Potentially fast progress towards proof-of-concept. However, clinical development can be complicated by the requirement to demonstrate the superiority of combination <i>versus</i> individual agents, as well as potentially increased risk of drug–drug interactions and formulation issues	Can be challenging to design a multiple ligand with the required ratio of activities and adequate selectivity at the discovery stage. However, the development program and regulatory approval process is the same as for a standard NCE
Intellectual property	Patent life of old drugs can be prolonged when combined with a new drug	Standard NCE position

## 19.3 Strategies and Challenges of Designing Multiple Ligands

Over the past two decades drug discoverers guided by the “one-target-one-disease” philosophy became quite proficient in developing highly selective ligands, even for targets where this was thought initially impossible, such as kinases and phosphodiesterases. Drug discovery platforms across the pharmaceutical industry evolved to fully support this paradigm, often leaving very little room for alternative approaches. However, this “one for all” approach has been challenged by the newly emerging multi-target drug discovery paradigm, which in terms of available tools, approaches and processes is still at a very early but rapidly developing stage. This section summarizes the current state of the art relating to the strategies and challenges in the field.

### 19.3.1 Medicinal Chemistry Strategies for DML Lead Generation

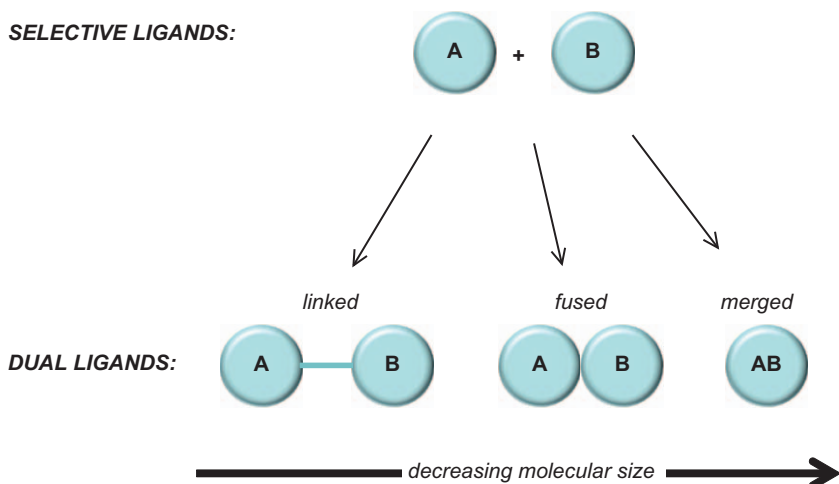
Two conceptually different approaches have dominated the lead generation strategy for a DML project: knowledge-based approaches that exploit information either from the general literature or from proprietary information from within an organization (Figure 19.1), and screening approaches that rely largely upon serendipity (Figure 19.2).<sup>8</sup>

#### 19.3.1.1 Knowledge-based Approaches

The knowledge-based approach, known as framework combination, starts with two compounds, each binding selectively to one of the targets. The initial goal is to “design in” both activities into a single lead molecule by combining the frameworks and the underlying pharmacophores of the two selective molecules (Figure 19.1). The intellectual elegance of the framework combination stems from the fact that often a wealth of SAR knowledge is on hand from previous selective ligand projects that can be used to guide the optimization process.

DMLs arising from framework combination can be viewed as linked, fused or merged depending upon the degree to which the frameworks have been integrated. In linked DMLs (conjugates), the molecular frameworks are not at all integrated and there is a distinct linker group between the two components that is not found in either of the selective ligands. Some linked DMLs contain a cleavable linker that is designed to be metabolized to release two ligands that interact independently with each target.<sup>11</sup> This scenario represents a half-way point between a true DML and a fixed dose combination. However, the linker is usually intended to be metabolically stable so that the single compound is capable of interacting with both targets, albeit different ends of the molecule may be responsible for the activity at the different targets.<sup>12</sup>

If the frameworks are essentially touching, so there is neither a discernable linker nor any framework overlap, the DML can be viewed as fused. In the

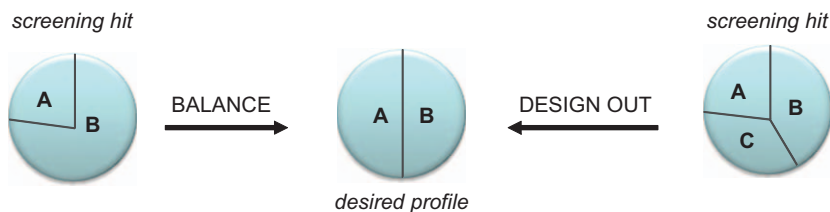


**Figure 19.1** Knowledge-based approaches: framework combination is a knowledge-based approach to generating DMLs. There is a continuum in the degree of merger of the frameworks of the target-selective starting ligands. In linked DMLs, the frameworks are connected *via* a definable linker, which in some cases is designed to be cleaved *in vivo* to release two independently acting drugs. In fused DMLs, the frameworks are directly attached and in the commonest form of DML, the frameworks are merged together.<sup>8</sup>

most common and most sought after type of DML, the frameworks are merged together by taking advantage of commonalities in the structures of the starting compounds. Medicinal chemists will normally aspire to maximize the degree of overlap in order to produce smaller and simpler molecules. The degree of framework combination for the examples reported in the literature forms a continuum, with high molecular weight (MW) DMLs with lengthy linker groups at one extreme, and small DMLs with highly merged frameworks at the other.

### 19.3.1.2 Screening Approaches

The screening of either diverse or focused compound libraries can deliver a single molecule that has at least minimal activity at each of the targets of interest. To date, there have not been many reported examples of DMLs derived *via* the HTS approach. This could be due to the logistical complications of screening against multiple targets in parallel or an inherently low probability of detecting a compound with a multiple profile of therapeutic interest from screening compounds at random. Due to the large number of compounds typically involved in diversity-based screening, they will usually be screened first at one target of interest and any actives will then be filtered on the basis of activity at the other target(s). Even if activity is observed for the second target,



**Figure 19.2** Screening approach: the screening of diverse or focused libraries can deliver a compound that has at least minimal activity at each target of interest. However it is unlikely that the hit compound has the optimal affinity for all targets so the profile must be balanced during optimization. Alternatively screening might deliver a compound that in addition to the desired activities has undesired activities and these must be designed out during optimization.<sup>8</sup>

usually the balance of affinities is non-optimal so the activity ratio must be adjusted during optimization.

Compared to HTS, there are many more examples in the literature of the screening of focused libraries of compounds selected from single-target projects or using prior knowledge of the targets. In focused screening, compound classes that are already known to be active against one of the targets of interest are screened against another target. For example, DMLs for kinase targets are usually discovered serendipitously through the cross-screening of ligands from selective kinase programmes against other kinases.<sup>13</sup> In addition to the desired activities, screening frequently provides hit compounds that bind to other targets, so to minimize the risk of side-effects the medicinal chemist will need to “design out” these undesired activities (Figure 19.2).<sup>14</sup>

### 19.3.1.3 Screening vs. Knowledge-based Approaches

The screening and framework combination approaches to lead generation have various advantages and disadvantages that influence which one is best applied to a particular project. A major advantage of the screening approach is that it starts from a compound that already has multiple activities built in, albeit these may be quite weak. Screening can add particular value if there is a lack of selective ligands for the targets of interest or little of the SAR information required for a knowledge-driven approach. Screening can deliver novel and unexpected chemotypes, sometimes providing hits for unusual target combinations that span unrelated receptor families. Since the framework combination strategy almost invariably produces dual ligands, discovering ligands that bind to more than two targets usually demands that a screening approach is followed. Screening can also provide ligands with improved physiochemical and pharmacokinetic (PK) properties compared to framework combination (see the section on physicochemical properties).

In the case of framework combination, incorporating a second activity into a compound that has no measurable affinity for that target, while retaining



affinity for the original target, is by no means an easy task. However, many literature examples testify to the fact that it can often be achieved by effectively leveraging SAR knowledge from historical selective ligand projects.<sup>8</sup> Although often resulting in molecules with sub-optimal physicochemical properties, the framework combination approach can provide rapid entry to conjugate molecules that may be used as intravenously (iv) administered drugs or biochemical tools, even for targets that are very different at the pharmacophore level (see the section on physicochemical properties). The chance of success with a random screening approach would be expected to rapidly diminish as the targets in a combination become more dissimilar. Given the added challenges of multiple ligand projects in general, it would make sense to employ both strategies if feasible to increase the overall chance of success.

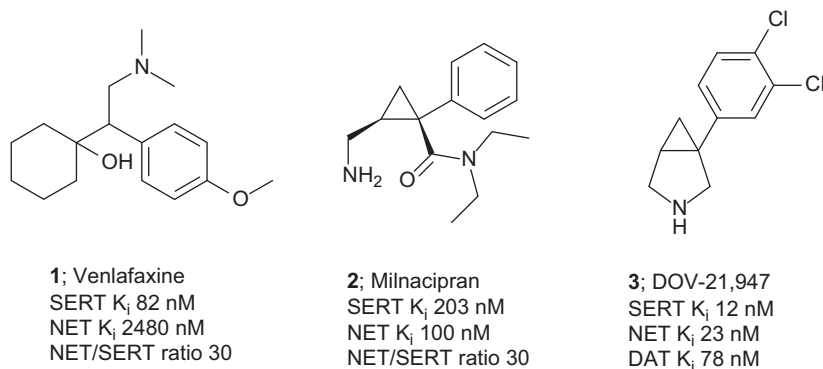
### 19.3.2 Medicinal Chemistry Challenges

No matter whether the lead compound is obtained by a screening or a framework combination approach, the ratio between desired activities and pharmacokinetic properties is likely to be sub-optimal. Thus, a medicinal chemist working on a DML lead optimization project is faced with two main challenges: balancing the desired while often having to remove undesired activities, and optimizing pharmacokinetic profile.

#### 19.3.2.1 Optimization of the Activity and Wider Selectivity Profile

Establishing what the desired level of modulation for each target should be for optimal efficacy and safety is not a straightforward task. Ideally, knowledge from clinical studies will guide researchers towards the optimal ratio, though for novel mechanisms of action, this clearly will not be available. In the absence of this knowledge, the aim of most historical DML projects has been to obtain the same degree of *in vitro* activity for each target, with the assumption that this will also lead to similar levels of enzyme modulation or receptor occupancy *in vivo*.

In the antidepressant field, the earliest efforts addressing serotonin reuptake inhibitors' (SSRIs') deficiencies, such as efficacy and time of onset of action, were focused on developing agents with potent and balanced activity at both serotonin and norepinephrine transporters (SERT and NET). The first antidepressant marketed as a serotonin and norepinephrine reuptake inhibitor (SNRI) was venlafaxine, **1**, launched in 1993 (Figure 19.3). Its success in the clinic where it demonstrated superior efficacy and earlier onset of action provided the proof of concept and for many years set the direction in drug discovery for the treatment of depression.<sup>15</sup> However, venlafaxine, has a 30-fold difference in *in vitro* potency at the two transporters, meaning it behaves as a multiple ligand *in vivo* only at high doses. Hence, the newer drugs such as milnacipran, **2**, have a more potent and balanced *in vitro* profile (Figure 19.3).<sup>16</sup>

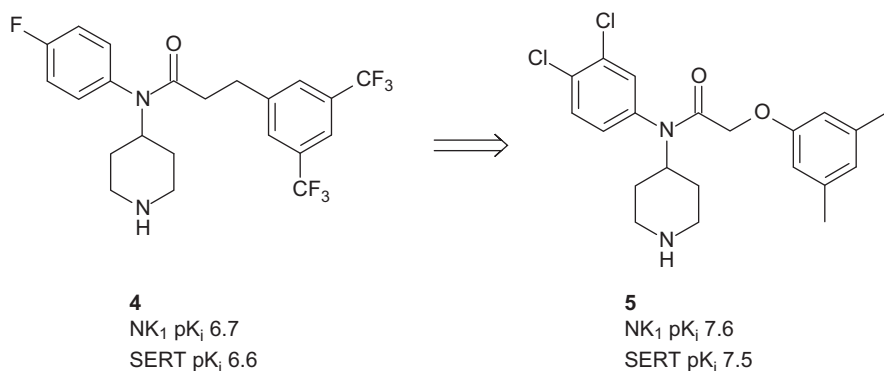


**Figure 19.3** Optimization of a DML profile to enhance efficacy and safety.

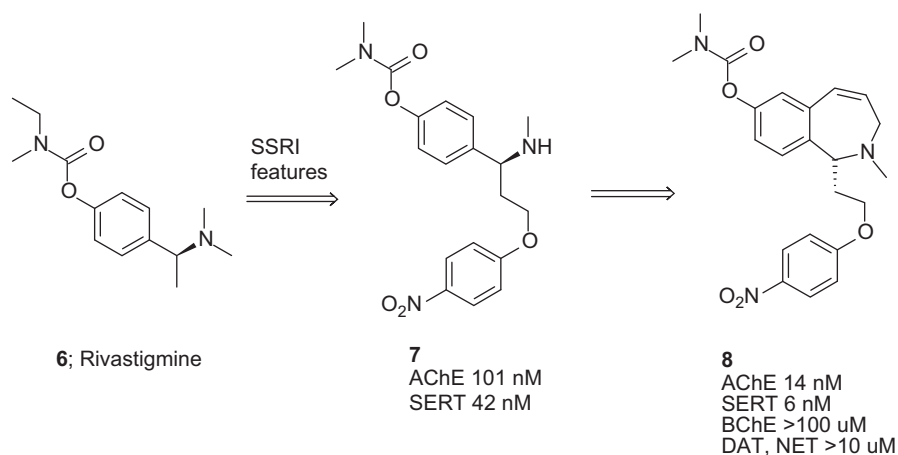
The vast majority of DMLs reported in the literature are dual ligands, which is probably not surprising considering that the design and optimization challenges increase dramatically as the number of targets to be balanced increases. Examples of DMLs with a more complex profile are mostly designed for targets that are closely related, such as combinations of monoamine transporters or monoamine GPCRs. For example, triple reuptake inhibitors (TRIs), such as DOV-21,247 (**3**, Figure 19.3),<sup>17</sup> which inhibit the dopamine transporter (DAT) as well as SERT and NET, are hoped to deliver better control of depression than either SSRIs or SNRIs (see Chapter 7).

It might be expected that deliberately designing a compound that selectively binds to two targets will be easy if those targets are closely related but will be particularly difficult if the targets belong to fundamentally different superfamilies, particularly if they do not share similar endogenous ligands. However, many literature examples show that spanning unrelated target families with a single drug-like molecule is not an impossible task, especially when employing screening strategy for hit identification. For example, an HTS campaign at UCB Pharma provided a multiple ligand with a surprising combination of activities at a peptide GPCR, the neurokinin  $NK_1$  receptor and SERT.<sup>18</sup> Although the hit **4** had only modest activity, optimization of each aromatic moiety in turn provided **5**, a more potent compound with balanced activity at both targets (Figure 19.4). As part of the optimization, an aryl ether moiety was introduced to reduce lipophilicity, providing physicochemical properties predictive of CNS penetration following oral administration. There is a close similarity between the initial hit and the optimized structure, illustrating the value of a high-quality hit when the receptor requirements are stringent.

There are also several examples where activity for distant targets and selectivity profiles can be achieved by rational approach. Kogen *et al.*<sup>19</sup> reported dual acetylcholinesterase (AChE)/SERT blockers such as **8**, which possess high selectivity over several closely related targets, including butyrylcholinesterase and the NET and DAT (Figure 19.5). A notable feature of this work is the



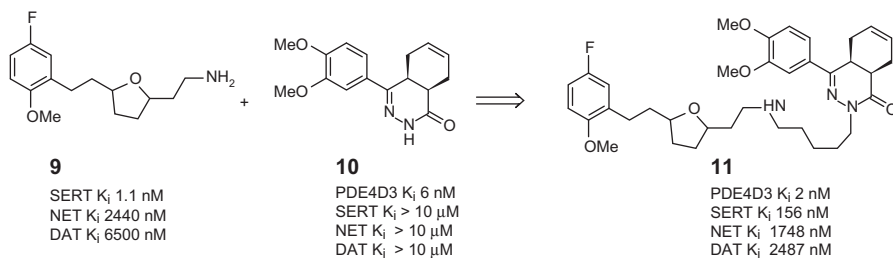
**Figure 19.4** Potent dual SERT/NK<sub>1</sub> receptor antagonist **5**, optimized from HTS hit **4**.<sup>18</sup>



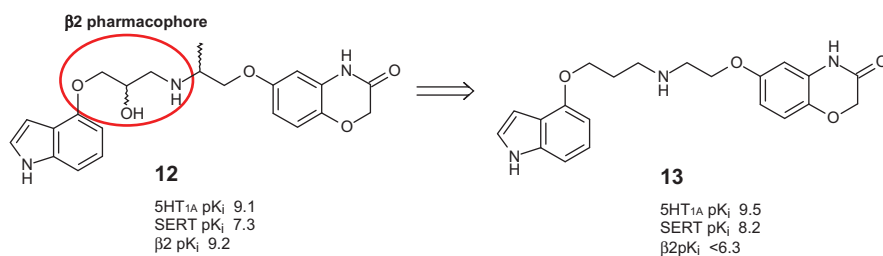
**Figure 19.5** A rationally designed dual AChE/SERT inhibitor with high selectivity over closely related targets.<sup>19</sup>

elegant use of biostructural information to guide the combination of the frameworks of rivastigmine (acetylcholine esterase, AChE, inhibitor) with the SSRI pharmacophore that provided dual inhibitor **7**. Conformational constraint using a 7-membered ring then gave compound **8**, with potent and balanced inhibition at the two diverse targets.

In their approach to the design of dual inhibitors for two distant targets such as SERT and phosphodiesterase 4 (PDE<sub>4</sub>), Cashman *et al.*<sup>20</sup> opted for the linking strategy (Figure 19.6). Indeed, the framework connection of two selective inhibitors *via* a five-carbon atom linker resulted in a potent and selective dual SERT/PDE<sub>4</sub> inhibitor, **11**. Their interest in this particular combination was based on preclinical data showing that selective PDE<sub>4</sub> inhibitors,



**Figure 19.6** Framework linking approach to dual SERT/PDE<sub>4</sub> inhibitors.<sup>20</sup>



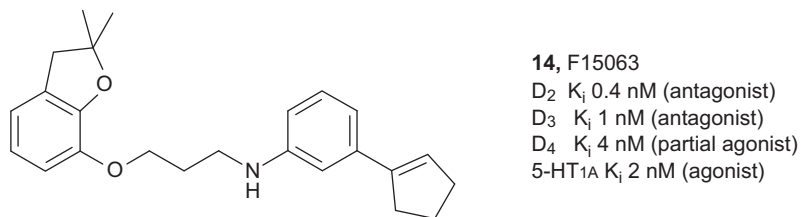
**Figure 19.7** Optimization of DMLs wider selectivity: dual SERT/5-HT<sub>1A</sub> ligands.<sup>14</sup>

*e.g.* rolipram, produce antidepressant-like effects in a number of preclinical models, such as the forced-swim test.<sup>21</sup>

The main reason for the linking strategy being rarely applied for CNS targets is that it normally leads to large and flexible ligands with compromised BBB permeability. Interestingly, **11** displayed significantly higher efficacy in the forced-swim test at 3 mg/kg dose (ip) than the SSRI or rolipram alone.<sup>20</sup> The reported mouse liver microsome data ( $T_{1/2}$  = 154 min.) and *in vivo* efficacy suggest better PK profile and brain exposure than one would expect from a molecule such as **11** with relatively high molecular weight (621 Da) and number of rotatable bonds (16). This example shows how the framework-linking approach can provide an effective access to proof-of-concept tools, especially for phylogenetically distant targets.

In addition to adjusting the ratio of activities, optimizing wider selectivity against a broad panel of targets can often be challenging. Atkinson *et al.*<sup>14</sup> successfully “designed out” adrenergic receptor  $\beta_2$  activity from a SERT inhibitor/5-HT<sub>1A</sub> antagonist, **12**, using effectively the knowledge of the anti-target pharmacophore (Figure 19.7).

Atypical antipsychotics often have a complex multi-receptor profile, and achieving the desired pharmacological profile without off-target activities associated with undesirable side-effects is often challenging, in particular if different primary target modalities are required, *e.g.* combining antagonist and agonist effect. Nevertheless, there are reports in the literature suggesting that



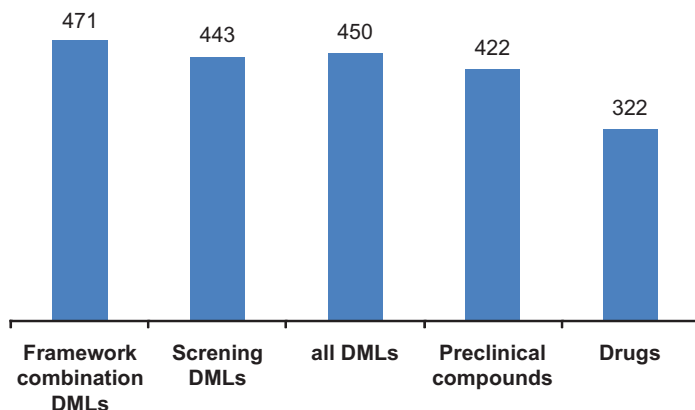
**Figure 19.8** Complex multi-receptor profile.<sup>22</sup>

this can be achieved by a rational design and selection process supported by an appropriate assay battery providing primary- and antitarget data in real time. For instance, Newman-Tancredi and coworkers were interested in identifying compounds with a  $D_2$  antagonist/ $5\text{-HT}_{1A}$  agonist receptor profile, which they hypothesized would produce a new generation of “atypical” antipsychotics with improved potential for the management of negative and cognitive symptoms of schizophrenia.<sup>22</sup> Antipsychotics that lack  $5\text{-HT}_{1A}$  receptor activation, such as haloperidol, are associated with extra-pyramidal symptoms (EPS) and lack of beneficial effect on negative symptoms, whereas excessive  $5\text{-HT}_{1A}$  receptor activation negates the  $D_2$  antagonism necessary for antipsychotic action,<sup>22,23</sup> hence the authors’ preference for ligands with  $5\text{-HT}_{1A}$  receptor agonism and 10–20-fold higher  $D_2$  receptor binding affinity. Whilst these two primary activities were critical, the authors allowed for additional activities if considered beneficial to the overall antipsychotic profile. This approach led to identification of F15063 (**14**, Figure 19.8), which, in addition to  $5\text{-HT}_{1A}$  agonism and potent  $D_2$  receptor antagonism, also displayed potent  $D_3$  receptor antagonism as well as  $D_4$  receptor partial agonism, both of which were considered beneficial by enhancing the compound’s potential effects on positive symptoms and procognitive deficits, respectively.

Importantly, the compound showed no interactions with targets associated with potential side-effects, including histaminergic and muscarinic receptors. F15063 was found to be active in *in vivo* models of positive and negative symptoms, and cognitive deficit of schizophrenia.<sup>24</sup>

### 19.3.2.2 The Physicochemical Challenge

An even greater challenge in MTDD than optimizing the balance of affinities and wider selectivity profile is to obtain DMLs displaying physicochemical and pharmacokinetic properties consistent with an oral drug. On average, the current generation of DMLs across all therapeutic areas have been found to be larger and more lipophilic than marketed drugs or preclinical compounds (Figure 19.9).<sup>25</sup> Larger and more lipophilic molecules are often associated with poorer oral absorption profiles, and yet this route of administration is desired for most DMLs. So optimizing the pharmacokinetics, in addition to attaining a balanced profile, can easily become the most challenging aspect of working with DMLs. One explanation for this has been the popularity of the



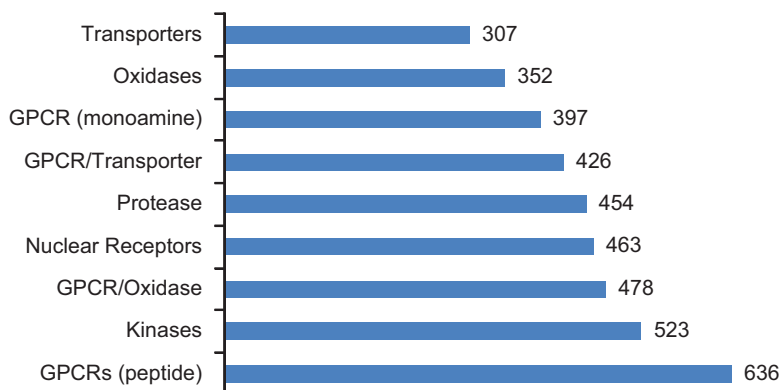
**Figure 19.9** The median molecular weight values for designed multiple ligands (DMLs) are higher than those for oral drugs or a general set of pre-clinical compounds.<sup>25</sup>

knowledge-based framework combination strategy, whereby the molecular frameworks from two selective ligands are combined. Given that the selective ligands used as the starting points are already drug-like in size and the extent to which the frameworks can be integrated is often low, this process can result in large property increases, which compromise oral bioavailability.

Nonetheless, the framework combination approach is a conceptually elegant knowledge-driven strategy that effectively uses SAR derived from selective ligand projects and, importantly, has led to oral drugs reaching the market, such as ziprasidone (Figure 19.17). To achieve an orally active DML, it is important that the degree of framework overlap is maximized and the size and complexity of the selective ligands is minimized. These goals will typically be more feasible for targets with simple endogenous ligands and conserved binding sites, such as monoamine GPCRs and transporters (Figure 19.16).

The MW for screening-derived DMLs is frequently lower than for the framework combination strategy, suggesting that this approach may provide a route to smaller and less complex leads (Figure 19.9). A starting compound obtained *via* screening already possesses multi-target activity to some extent. During optimization, the activities are usually balanced by adding modestly sized groups or modifying the existing functionality. This typically had less of an effect on the overall size and physicochemical properties of the molecule than the combination of two frameworks.

Over recent years, there has been an increasing amount of evidence that physicochemical properties are less favourable for the ligands from some proteomic target families of interest in drug discovery than for others, making the discovery of orally active drugs for those targets more challenging. Similar trends amongst the target families have also been reported for DMLs. The target family that has consistently given the highest physicochemical property values for both preclinical compounds and DMLs is the peptide GPCRs



**Figure 19.10** Median MW of DMLs classified according to proteomic target family.<sup>25</sup>

(Figure 19.10). For example, DMLs for peptide GPCRs have a median MW of 636 and a median cLogP of 5.1, figures in excess of those defined in the “rule of five” for drug-likeness.<sup>26</sup> At the other end of the spectrum, the ligands for transporters, monoamine GPCRs and oxidases generally possess favourable physicochemical properties and the feasibility of such targets for DML projects using a variety of lead discovery strategies will be relatively high.<sup>25</sup>

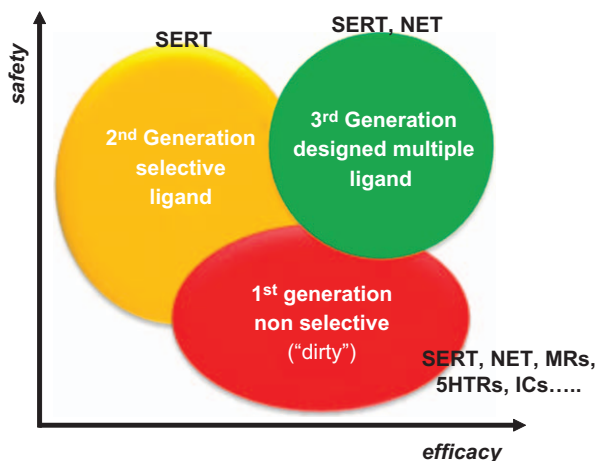
The analysis indicates that designing DMLs for peptide GPCRs will be a more difficult endeavour than for other types of GPCR or indeed for selective ligands for individual peptide GPCRs. In such cases, a strong emphasis is often required during lead optimization on simplifying the structure of the lead compound.

In a number of literature examples, the combination of a desirable *in vitro* profile with the pharmacokinetic profile required for the development of an oral drug was not achieved. Where the pharmacophores are fundamentally different, it may not be possible to integrate the requirements of both binding sites into a small, compact molecule and a higher MW compound may be unavoidable. Inevitably, this will mean that some combinations of targets will be more difficult, if not impossible, to address with a drug-like molecule.

## 19.4 Multi-target Drug Discovery for Psychiatric Disorders – Case Studies

A survey of the primary medicinal chemistry literature suggests that psychiatry is one of the most common therapeutic areas where MTDD is employed. Within psychiatry, similarly to a number of other therapeutic areas, drug discovery has historically been characterized by a three-stage evolution, from non-selective drugs displaying undesirable side-effects, followed by highly specific drugs with improved safety profile but lower efficacy, to DMLs with more optimal balance of efficacy and safety.





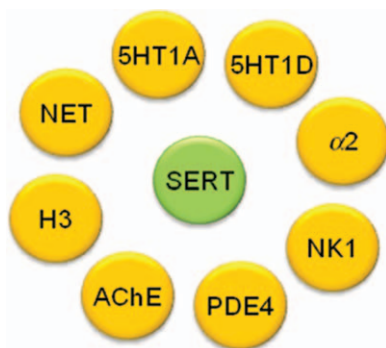
**Figure 19.11** The three-stage evolution of antidepressant drugs.

For example, in order to improve the side-effect profile of clozapine, an atypical antipsychotic displaying a highly complex *in vitro* pharmacology, a number of ligands selective for single receptors targeted by the drug have been developed over the past two decades, *e.g.* 5-HT<sub>2A</sub> and D<sub>4</sub> antagonists.<sup>27</sup> However, this reductionist approach has yet to produce a compound with comparable clinical efficacy. In fact, since it is now widely accepted that activity at a single receptor is not sufficient to convey clozapine's atypical profile, the research has recently shifted towards ligands with combined activities at two or more specific receptors believed to be related to the symptomatology of psychosis, *e.g.* D<sub>2</sub>/5-HT<sub>2A</sub>.<sup>28,29</sup>

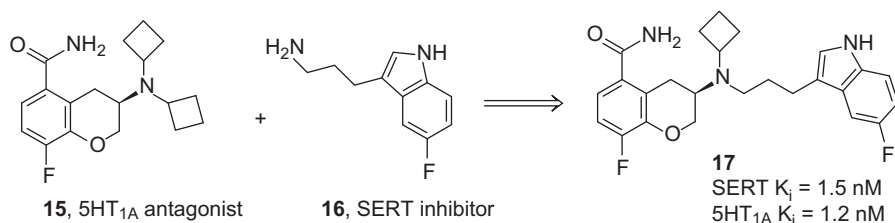
Similarly, the introduction of SSRIs with markedly improved side-effect profile in comparison with the previous generation of tricyclic antidepressants (TCAs) was a major breakthrough in terms of safe management of depression (Figure 19.11). However, SSRIs showed very little improvement in terms of the onset of action, and like TCAs require 4–6 weeks to establish full therapeutic efficacy. To address this deficiency, a new generation of dual agents has been developed: serotonin norepinephrine reuptake inhibitors (SNRI). Indeed, venlafaxine, one of the first SNRIs, shows both a faster onset of action and increased clinical efficacy.<sup>30</sup> Currently, the most common theme in terms of the design of multiple action ligands is to take a well-validated target and add one or more target activities aiming to enhance efficacy and/or reduce side-effects.

#### 19.4.1 Depression: SERT-plus Combinations

To address the SSRI deficiencies related to efficacy or time of onset, industry and academia have been seeking to supplement SERT inhibition with additional activity on other targets, some of which are depicted in Figure 19.12.



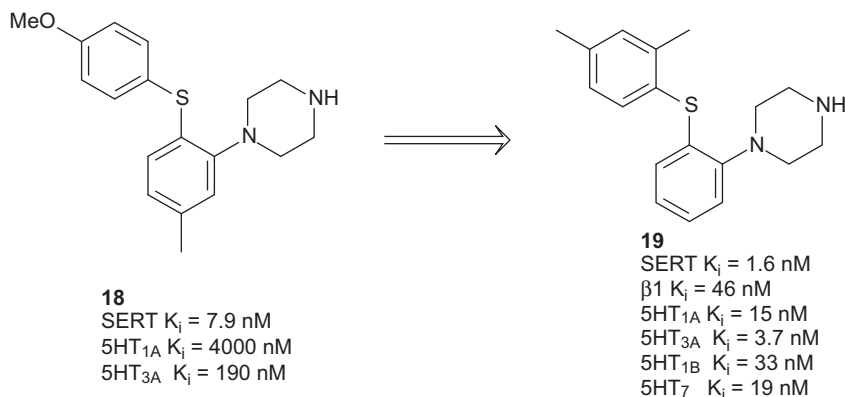
**Figure 19.12** “SERT plus” strategy: combining SERT inhibition with activity on another target is a common theme in antidepressant drug discovery. This figure highlights some of the SERT partner targets reported in the literature.



**Figure 19.13** Framework merging strategy: dual SERT/5-HT<sub>1A</sub> inhibitors.<sup>31</sup>

One of the most widely accepted hypotheses is that the delayed time of onset is the result of the 5-HT<sub>1A</sub> auto-receptors becoming desensitized by sustained SERT blockade. Therefore, by inhibiting 5-HT<sub>1A</sub> receptors, the onset time might be accelerated. Hatzenbuehler and his colleagues employed the framework combination strategy to design a dual SERT inhibitor/5-HT<sub>1</sub> antagonist starting from corresponding selective ligands (Figure 19.13).<sup>31</sup> The presence of a basic nitrogen was the common pharmacophoric feature that allowed the two frameworks to be merged resulting in **17** with a potent and balanced dual profile, and >100-fold selectivity over other biogenic amine receptors and transporters. An oral microdialysis study (30 mg/kg) has shown that this compound acutely elevates serotonin levels in the rat frontal cortex to a similar extent of chronic (14 day) SSRI treatment, consistent with “rapid onset” antidepressant activity.

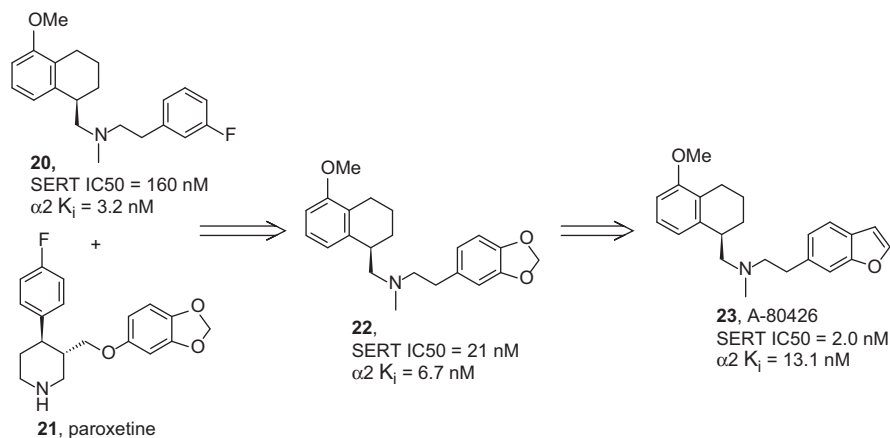
An alternative approach in addressing the delayed time of onset is to “speed up” the desensitization of 5-HT<sub>1A</sub> receptors by their direct activation. Indeed, it has been demonstrated in the clinic that antidepressant activity of SSRIs can be augmented by co-administration of pindolol, which was attributed to its partial agonistic effect on 5-HT<sub>1A</sub> receptors.<sup>32</sup> Moreover, scientists at Lundbeck were



**Figure 19.14** SERT/5-HT<sub>1A</sub>/5-HT<sub>3</sub> antagonists derived from the screening approach.<sup>33</sup>

interested to supplement the dual SERT inhibition/5-HT<sub>1A</sub> receptors agonism profile with antagonism of the 5-HT<sub>3</sub> receptors (Figure 19.14).<sup>33</sup> The rationale for the additional activity was based on preclinical data showing that 5-HT<sub>3</sub> antagonists eliminate the inhibitory tonus of 5-HT<sub>3</sub> receptors on the release of norepinephrine and acetylcholine in the forebrain, and therefore suggesting a possible positive effect on mood and cognitive impairment in patients with depression.<sup>34</sup> Additionally, it has been demonstrated that the 5-HT<sub>3</sub> receptor antagonist odansetron potentiates citalopram-induced increases in serotonin levels in rat brain.<sup>35</sup> Anticipating challenges with identification of molecules that differently modulate three targets from three different gene families (a monoamine transporter inhibitor, GPCR agonist and ion channel blocker) it was accepted from the onset of the program that compounds fulfilling the design hypothesis might well be endowed with additional pharmacology.

A focused screening campaign led to discovery of arylpiperazine **18** displaying potent SERT inhibition, very weak 5-HT<sub>1A</sub> and moderate 5-HT<sub>3</sub> receptor affinities, as well as high 5-HT<sub>1C</sub> and  $\alpha_1$  receptor binding, with  $K_i$  values <100 nM. The SAR exploration and multi-parameter optimization efforts to improve the *in vitro* pharmacology, ADME and safety profile of **18** led to the identification of **19**, which in a broad selectivity panel of assays displayed high affinity for SERT, noradrenergic  $\beta_1$  and serotonergic 5-HT<sub>1B</sub> and 5-HT<sub>7</sub>, as well as modest binding at histamine H<sub>2</sub> ( $K_i$  = 180 nM), noradrenergic  $\beta_2$  ( $K_i$  = 560 nM), serotonergic 5-HT<sub>1C</sub> ( $K_i$  = 180 nM) and 5-HT<sub>6</sub> ( $K_i$  = 330 nM) receptors (Figure 19.14). Importantly, the compound displayed high selectivity against key antitargets such as dopamine transporter (DAT  $K_i$  = 890 nM) and CYPs (1A2, 2C9, 2D6 and 3A4 >10  $\mu$ M). In rat *in vivo* microdialysis experiments, three days of treatment with **19** (5 or 10 mg/kg/day) resulted in significant increases in extra-cellular serotonin levels at low SERT occupancy, which is in contrast to SSRIs where chronic treatment is generally required to overcome the inhibitory feedback mechanisms and produce a



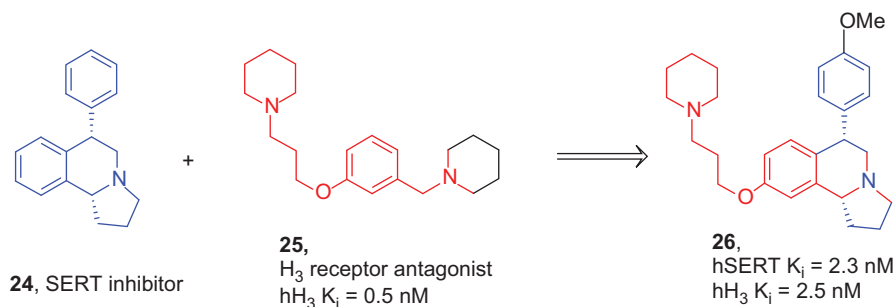
**Figure 19.15** Fused DML approach: dual SERT/ $\alpha_2$  ligands.<sup>37</sup>

positive serotonin response in preclinical models. This compound is currently in phase III clinical trials for major depressive disorder.<sup>33</sup>

Since the synaptic release of serotonin is inhibited not only by pre-synaptic 5-HT<sub>1A</sub> auto-receptors but also by  $\alpha_2$ -heteroreceptors,<sup>36</sup> Meyer and coworkers from Abbott were interested in developing dual SERT inhibitor/ $\alpha_2$  receptor antagonists (Figure 19.15).<sup>37</sup> Their search for a ligand with this profile began with screening hit **20** possessing high affinity for the  $\alpha_2$  receptor ( $K_i$  = 3.2 nM) and modest potency at inhibiting serotonin uptake (IC<sub>50</sub> = 160 nM).

In order to enhance SERT potency the authors elected to incorporate into **20** structural features common to other SERT inhibitors from the literature. Molecular modelling indicated that good overlap could be achieved between the nitrogen and the (methylenedioxy)phenyl of potent uptake inhibitor paroxetine **21** and the nitrogen and fluorophenyl of **20**. Indeed, the hybrid analogue **22** displayed 8-fold greater SERT potency (IC<sub>50</sub> = 21 nM) and nearly equivalent  $\alpha_2$  binding affinity ( $K_i$  = 6.7 nM). SAR studies around **22** indicated that the left-hand portion of the molecule is not at all tolerant to structural modifications, so that the further optimization efforts focused on the more tolerant right-hand side. The structural flexibility in this portion of the molecule allowed for optimization of physicochemical and ADME properties, which resulted in the discovery of **23** (A-80426), showing a potent and balanced profile ( $\alpha_2$   $K_i$  = 2.0 nM, SERT IC<sub>50</sub> = 13.1 nM). However, despite its favourable *in vitro* pharmacological and ADME profile, **23** was not an effective  $\alpha_2$  blocker *in vivo* and was proved inactive in the behavioural despair models of depression.<sup>38</sup>

Patients with depressive disorders often suffer from cognitive impairment and fatigue, which may persist even after remission. SSRIs frequently fail to improve these symptoms, even as mood improves, and some would even induce fatigue and excessive sleepiness. Keith and colleagues at Johnson & Johnson proposed that a dual SERT inhibitor/histamine H<sub>3</sub> receptor antagonist may



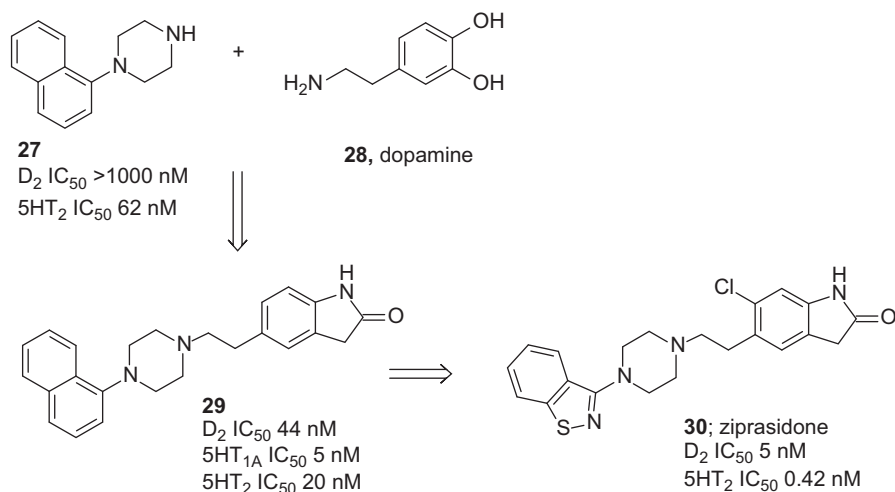
**Figure 19.16** Framework merging example: dual SERT/ $H_3$  antagonist.<sup>39</sup>

address these therapeutic needs.<sup>39</sup>  $H_3$  receptor antagonists are shown in a variety of animal models to improve cognition, increase wakefulness and decrease REM sleep, which has been observed to improve the mood of depressed patients in sleep-deprivation studies. To create a starting point, the group elected to merge the selective SERT framework from an earlier in-house program with an  $H_3$  receptor antagonist pharmacophore, a linear disposition of two tertiary amines separated by a 3- or 4-substituted phenylene and a hydrophobic chain of at least four atoms (Figure 19.16).

Indeed, this approach led to the discovery of pyrrolidino-tetrahydroisoquinolines such as **26**, displaying both potent human SERT inhibition and  $H_3$  receptor antagonism,  $K_i$  2.3 nM and 2.5 nM, respectively. Compound **26** showed modest oral bioavailability in rat (16%) and slow absorption and subsequently slow elimination from the brain ( $C_{max} = 1.28 \mu\text{M}$  at 24 h), presumably due to its dibasic character. Despite the modest bioavailability (offset by high brain concentration and potency) the compound showed robust and sustained pharmacology in the mouse 5-hydroxytryptophan potentiated (5-HTP) head twitch model of SERT inhibition and significant increase of serotonin and dopamine in the frontal cortex of the rat brain as measured by *in vivo* microdialysis.<sup>39</sup>

### 19.4.2 Schizophrenia: Ziprasidone and Other Antipsychotic Agents

Schizophrenia has traditionally been treated with  $D_2$  antagonists such as haloperidol. Whilst demonstrating efficacy against the positive symptoms of the disease (hallucinations, delusions), haloperidol does not address the negative symptoms (such as social withdrawal) and causes side motor effects known as extra-pyramidal syndrome (EPS).<sup>40</sup> The “atypical” antipsychotic drug clozapine addresses both positive and negative symptoms without producing EPS. One possible explanation for this atypical profile is that clozapine has higher antagonist affinity for the 5-HT<sub>2</sub> receptor than it does for the  $D_2$  receptor.<sup>41</sup> Several so-called atypical antipsychotics with low  $D_2/5\text{-HT}_2$  binding ratios have been introduced into the market (see Chapters 2 and 3).



**Figure 19.17** Framework fusing strategy: discovery of ziprasidone.<sup>42</sup>

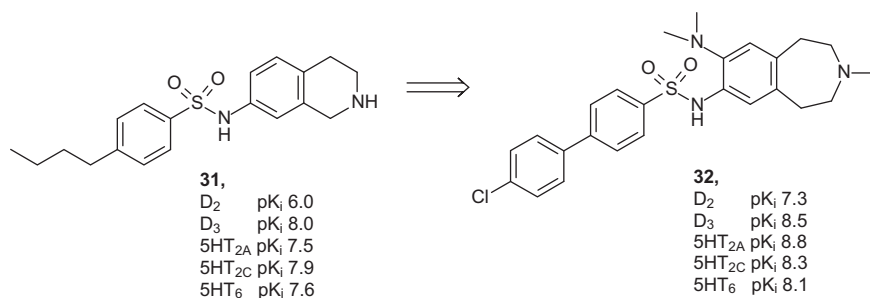
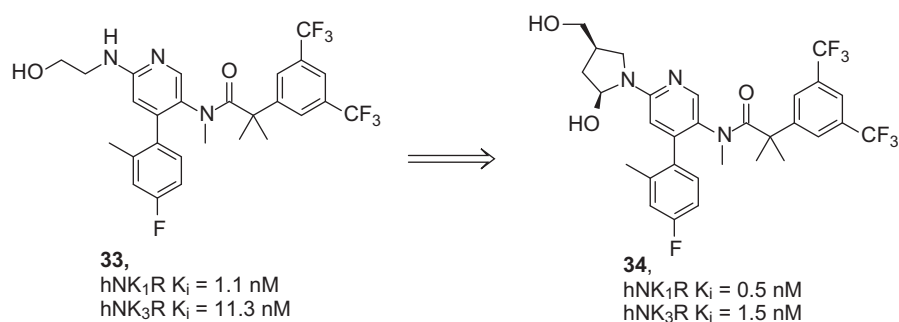
To obtain a dual  $D_2/5\text{-HT}_2$  antagonist, Lowe *et al.* at Pfizer used a framework combination approach and fused the structures of a 5-HT selective ligand **27** and dopamine **28** (Figure 19.17).<sup>42</sup> Interestingly, this structural change resulted in conversion of dopamine into a potent  $D_2$  antagonist. Since the starting ligands are small, the resulting fused DML has a relatively low MW of 371 Da. A heterocyclic biosisotere mimicking one of dopamine OH groups was introduced to give **29**. Further optimization involved replacing the naphthyl group by a 1,2-benzisothiazole group (**30**), which provided a desirable  $D_2/5\text{-HT}_2$  activity ratio of 11, comparable to the atypical agent, clozapine. The  $D_2/\alpha 1$  ratio of 0.44 for **30** is substantially lower than that for clozapine, suggesting the former should have a lower propensity to cause orthostatic hypotension. Indeed, following positive clinical outcome ziprasidone (**30**) was launched in 2001 by Pfizer for the treatment of schizophrenia.

Atypical schizophrenia drugs usually have a multitude of other activities in addition to  $D_2$  and  $5\text{-HT}_2$  antagonism. An analysis of the receptor interaction profile of marketed antipsychotics<sup>43</sup> led Garzya *et al.* to aim for a molecule that selectively blocks five receptors deemed to have therapeutic value, the  $D_2$ ,  $D_3$ ,  $5\text{-HT}_{2A}$ ,  $5\text{-HT}_{2C}$  and  $5\text{-HT}_6$  receptors (Table 19.2).<sup>44</sup> They also had a list of ten receptors that they wanted to avoid, such as the  $H_1$  receptor, as well as at other monoamine targets,  $\alpha_{1B}$ ,  $M_{1-4}$  and  $\beta_{1-3}$ .

By focused screening they discovered hit **31** that blocked the five desired targets (Figure 19.18). The systematic SAR effort produced a DML **32** with the optimal balance of affinities and critically, showing no the undesired cross-reactivity, which is remarkable considering challenges in designing such a complex pharmacological profile. In this respect, synthetic feasibility is an important consideration when assessing DML hits, since rapid synthesis/test iterations are often crucial to ultimate success. The compound was further

**Table 19.2** Desired target profile.<sup>44</sup>

Receptor	pK <sub>i</sub>	Beneficial effect
D <sub>2</sub>	7–8	Effect on positive symptoms. Lower affinity was chosen to minimize the risk of D <sub>2</sub> -mediated side effects that occur at higher levels of receptor occupancy, <i>e.g.</i> EPS and hyperprolactinemia.
D <sub>3</sub>	>8	Effect on positive symptoms.
5-HT <sub>2A</sub>	>8	Contributes to atypical antipsychotic profile.
5-HT <sub>2C</sub>	>8	Counteracts D <sub>2</sub> -mediated EPS. Also produces anxiolytic and antidepressant effects.
5-HT <sub>6</sub>	>8	Addresses cognitive deficits.

**Figure 19.18** Screening approach to a complex antipsychotic profile.<sup>44</sup>**Figure 19.19** Dual NK<sub>1</sub>/NK<sub>3</sub> receptor antagonists for the treatment of schizophrenia.<sup>45</sup>

profiled *in vivo*, where it showed activity in amphetamine-induced hyperactivity model (ED<sub>50</sub> 20.6 mg/kg, po, rat), whilst showing no propensity to induce catalepsy in rats up to 100 mg/kg.<sup>44</sup>

Not all approaches to the treatment of schizophrenia are focused on targeting dopamine and/or serotonin receptors. For example, Hoffmann and coworkers at Hoffman-La Roche reported dual neurokinin-1 (NK<sub>1</sub>) and neurokinin-3 (NK<sub>3</sub>) receptor antagonists that are expected to address both positive and negative symptom domains, while having improved side-effect profile



compared to the current standard of care (Figure 19.19).<sup>45</sup> The initial lead **33**, discovered serendipitously from the internal selective NK<sub>1</sub> receptor antagonist program, displayed high human NK<sub>1</sub> and an order of magnitude lower affinity for human NK<sub>3</sub> receptors, 1.1 nM and 11.3 nM, respectively.

The authors hypothesized that the hydroxyethylamine substituent, a unique feature of **33** compared to related NK<sub>1</sub> receptor selective ligands, is a pharmacophoric requirement for NK<sub>3</sub> receptor binding. Therefore, optimization efforts focused on modifications around that region of the molecule, which ultimately led to the discovery of potent and well-balanced NK<sub>1</sub>R/NK<sub>3</sub>R antagonists such as **34**. In preliminary evaluation of *in vivo* properties, orally administered **34** inhibited NK<sub>1</sub> agonist-induced foot tapping behaviour in gerbils with an ID<sub>50</sub> of 1.3 mg/kg, demonstrating that its molecular properties are suitable to reach the intended target in the CNS and elicit the expected pharmacodynamic response at a relatively low dose.<sup>45</sup>

## 19.5 Summary and Outlook

The past two decades in pharmaceutical industry were marked by high attrition rates, increased cost and incremental improvements. There has been a growing acceptance in recent years that the next generation of breakthrough therapies will only be possible if it is recognized that the complex polygenic aetiology that characterizes many diseases requires more complex solutions than those derived from the currently predominant “one-target-one-disease” paradigm. Indeed, numerous reports in the literature suggest that agents acting at multiple therapeutic targets could have superior efficacy and safety profile compared to those highly selective for a single target. Consequently, there is an ever-increasing interest in designed multiple ligands (DMLs) and multi-target drug discovery (MTDD) in general. Many elegant, and increasingly rational, approaches to the discovery of DMLs have been reported in the literature, of which only a small selection has been described above.

The main challenges in the design and optimization of DMLs are related to achieving balanced and potent desired activities whilst maintaining wider selectivity, and optimizing pharmacokinetic profile. It is possible, however, that multi-target acting ligands may reduce the need for the high-affinity binding that is characteristically required for target-selective agents. Where synergy exists between two or more targets, it is conceivable that multiple ligands with only relatively modest activity at two or more targets might still produce superior *in vivo* effects, compared to higher affinity target-selective compounds. Indeed, the work by Csermely *et al.*<sup>46</sup> employing biological network models of anti-microbial drugs has suggested that partial inhibition of a number of targets can be more effective than complete inhibition of a single target. Although more specific network models will be needed to examine how effective weak binders might be in areas such as psychiatric disorders, the preclinical and clinical data are encouraging. For example, it has been shown that low dose of atypical antipsychotics such as risperidone are effective augmentation agents in treatment of depressed patients unresponsive to SSRIs.<sup>47</sup> Integration of such

advanced clinical information with ever-expanding understanding of the brain signalling pathways and circuits will enable construction of robust biological network models and ultimately a rational design of optimal target combinations that are capable of efficiently restoring the equilibrium of an altered disease state.<sup>48</sup>

In order to address the “physicochemical challenge”, new design strategies will be needed, such as recently proposed fragment-based approaches.<sup>49</sup> Given the promiscuity of fragment-sized molecules,<sup>50</sup> typically <250 Da, screening of a fragment library could be an effective approach to core scaffolds binding with high ligand efficiency to desired targets. Considering importance of bio-structural information for the success of fragment optimization, this approach is limited mostly to crystallographically enabled targets, such as proteases, phosphodiesterases and kinases. However, given the current advances in crystallography, the scope and diversity of applicable targets will continue to expand rapidly, and even include traditionally difficult membrane-bound proteins, such as GPCRs.<sup>51</sup>

In summary, MTDD targeting biological network states rather than individual proteins allied with a fragment-based approach to DMLs may represent a powerful new paradigm for deriving the next-generation drugs for the treatment of psychiatric disorders with improved efficacy and safety profiles.

## References

1. A. Mullard, *Nat. Rev. Drug Discovery*, 2012, **11**, 91.
2. I. Kola and J. Landis, *Nat. Rev. Drug Discovery*, 2004, **3**, 711.
3. B. L. Roth, D. J. Sheffler and W. K. Kroeze, *Nat. Rev. Drug Discovery*, 2004, **3**, 353.
4. J. R. Morphy, C. Kay and Z. Rankovic, *Drug Discovery Today*, 2004, **9**, 641–651.
5. J. R. Morphy and Z. Rankovic, *Curr. Pharm. Des.*, 2009, **15**, 587.
6. S. Englisch and M. Zink, *Drug Discovery Today: Ther., Strategies*, 2011, **8**, 17.
7. M. J. Keiser, V. Setola, J. J. Irwin, C. Laggner, A. I. Abbas, S. J. Hufeisen, N. H. Jensen, M. B. Kuijer, R. C. Matos, T. B. Tran, R. Whaley, R. A. Glennon, J. Hert, K. L. H. Thomas, D. D. Edwards, B. K. Shoichet and B. L. Roth, *Nature*, 2009, **462**, 175.
8. J. R. Morphy and Z. Rankovic, *J. Med. Chem.*, 2005, **48**, 6523.
9. S. Frantz, *Nat. Rev. Drug Discovery*, 2006, **5**, 881.
10. R. Osborne, N. Clarke, P. Glossop, A. Kenyon, H. Liu, S. Patel, S. Summerhill and L. H. Jones, *J. Med. Chem.*, 2011, **54**, 6998.
11. M. Di Napoli and F. Papa, *Curr. Opin. Invest. Drugs*, 2003, **4**, 1126.
12. S. Zhang, A. Yekkiralala, Y. Tang and P. Portoghese, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6978.
13. J. R. Morphy, *J. Med. Chem.*, 2010, **53**, 1413.
14. P. Atkinson, S. Bromidge, M. Duxon, M. Laramie, L. Gaster, M. Hadley, B. Hammond, C. Johnson, D. Middlemiss, S. North, G. Price, H. Rami,

- J. Riley, C. Scott, T. Shaw, K. Starr, G. Stemp, K. Thewlis, D. Thomas, M. Thompson, A. Vong and J. Watson, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 737.
15. S. M. Stahl, *Biol. Psychiatry*, 2002, **52**, 1166.
16. M. Briley, J. F. Prost and C. Moret, *Int. Clin. Psychopharmacol.*, 1996, **11**(4), 9.
17. P. Skolnick, P. Popik, A. Janowsky, B. Beer and A. S. Lippa, *Eur. J. Pharmacol.*, 2003, **461**, 99.
18. T. Ryckmans, L. Balançon, O. Berton, C. Genicot, Y. Lamberty, B. Lallemand, P. Pasau, N. Pirlot, L. Quéré and P. Talaga, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 261.
19. N. Toda, K. Tago, S. Marumoto, K. Takami, M. Ori, N. Yamada, K. Koyama, S. Naruto, K. Abe, R. Yamazaki, T. Hara, A. Aoyagi, Y. Abe, T. Kaneko and H. Kogen, *Bioorg. Med. Chem.*, 2003, **11**, 4389.
20. J. R. Cashman, T. Voelker, H.-T. Zhang and J. M. O'Donnell, *J. Med. Chem.*, 2009, **52**, 1530.
21. M. D. Houslay, P. Shafer and K. Y. Zhang, *Drug Discovery Today*, 2005, **10**, 1503.
22. A. Newman-Tancredi, M. B. Assié, J. C. Martel, C. Cosi, L. B. Slot, C. Palmier, I. Rauly-Lestienne, F. Colpaert, B. Vacher and D. Cussac, *Br. J. Pharmacol.*, 2007, **151**, 237.
23. M. B. Assie, V. Ravailhe, V. Faucillon and A. Newman-Tancredi, *J. Pharmacol. Expt. Ther.*, 2005, **315**, 265.
24. R. B. Depoortere, A. Auclair, L. Bruins-Slot and A. Newman-Tancredi, *Int. J. Neuropsychopharmacol.*, 2006, **9**(1), P01.165.
25. J. R. Morphy and Z. Rankovic, *J. Med. Chem.*, 2006, **49**, 4961.
26. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3.
27. M. S. Kramer, B. Last, A. Getson and S. A. Reines, *Arch. Gen. Psychiatry*, 1997, **54**, 567.
28. M. Rowley, L. J. Bristow and P. H. Hutson, *J. Med. Chem.*, 2001, **44**, 477.
29. S. R. Marder, B. Roth, P. F. Sullivan, E. M. Scolnick, E. J. Nestler, M. A. Geyer, D. R. Welnberger, M. Karayiorgou, A. Guidotti, J. Gingrich, S. Akbarian, R. W. Buchanan, J. A. Lieberman, P. J. Conn, S. J. Haggarty, A. J. Law, B. Campbell, J. H. Krystal, B. Moghaddam, A. Saw, M. G. Caron, S. R. George, J. A. Allen and M. Solis, *Ann. NY Acad. Sci.*, 2011, **1236**, 30.
30. S. M. Stahl, R. Entsuah and R. L. Rudolph, *Biol. Psychiatry*, 2002, **52**, 1166.
31. N. T. Hatzenbuehler, D. A. Evrard, B. L. Harrison, D. Huryn, J. Inghrim, C. Kraml, J. F. Mattes, R. E. Mewshaw, D. Zhou, G. Hornby, Q. Lin, D. L. Smith, K. M. Sullivan, L. E. Schechter, C. E. Beyer and T. H. Andree, *J. Med. Chem.*, 2006, **49**, 4785.
32. F. Artigas and A. Adell, *Curr. Drug Targets*, 2006, **7**, 139.
33. B. Bang-Anderson, T. Ruhland, M. Jorgensen, G. Smith, K. Fredeirksen, K. G. Jensen, H. Zhong, S. M. Nielsen, S. Hogg, A. Mork and T. B. Stensbol, *J. Med. Chem.*, 2011, **54**, 3206.

34. Z. Yan, *Mol. Neurobiol.*, 2002, **26**, 203.
35. R. Bergeron and P. Blier, *Am. J. Psychiatry*, 1994, **152**, 1084.
36. T. J. Feuerstein and M. Gothert, *J. Neurochem.*, 1993, **61**, 474.
37. M. D. Meyer, A. A. Hancock, K. Tietje, K. B. Sippy, R. Prasad, D. M. Stout, D. L. Arendsen, B. G. Donner and W. A. Carroll, *J. Med. Chem.*, 1997, **40**, 1049.
38. W. J. Giardina, S. A. Buckner, M. E. Brune, A. A. Hancock, C. T. Wismer, I. Milicic, A. J. Rattin, S. Roux, J. Wettstein, M. D. Mayer, R. D. Porsolt, J. F. Kerwin, Jr and M. Williams, *Drug. Dev. Res.*, 1995, **35**, 246.
39. J. M. Keith, L. A. Gomez, R. L. Wolin, A. J. Barbier, S. J. Wilson, J. D. Boggs, C. Mazur, I. C. Fraser, B. Lord, L. Aluisio, T. W. Lovenberg and N. I. Carruthers, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 2603.
40. J. Crilly, *History of Psychiatry*, 2007, **18**, 39.
41. H. Y. Meltzer, *Neuropsychopharmacology*, 1999, **21**, 106.
42. J. Lowe, T. Seeger, A. Nagel, H. Howard, P. Seymour, J. Heym, F. Ewing, M. Newman and A. Schmidt, *J. Med. Chem.*, 1991, **34**, 1860.
43. A. I. Graul, *Drugs Future*, 2003, **28**, 1103.
44. V. Garzya, I. T. Forbes, A. D. Gribble, M. S. Hadley, A. P. Lightfoot, A. H. Payne, A. B. Smith, S. E. Douglas, D. G. Cooper, I. G. Stansfield, M. Meeson, E. E. Dodds, D. N. Jones, M. Wood, C. Reavill, C. A. Scorer, A. Worby, G. Riley, P. Eddershaw, C. Ioannou, D. Donati, J. J. Hagan and E. A. Ratti, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 400.
45. J.-U. Peters, T. Hoffmann, P. Schnider, H. Stadler, A. Koblet, A. Alker, S. M. Poli, T. M. Ballard, W. Spooren, L. Steward and A. J. Sleight, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 3405.
46. P. Csermely, V. Agoston and S. Pongor, *Trends in Pharmacological Science*, 2005, **26**, 178.
47. K. Rasmussen, *Drug Discovery Today*, 2006, **11**, 623.
48. A. D. W. Boran and R. Lyengar, *Curr. Opin. Drug Discovery Dev.*, 2010, **13**, 297.
49. J. R. Morphy and Z. Rankovic, *Drug Discovery Today*, 2007, **12**, 156.
50. A. L. Hopkins, J. S. Mason and J. P. Overington, *Curr. Opin. Struct. Biol.*, 2006, **16**, 127.
51. D. M. Rosenbaum, S. G. F. Rasmussen and B. K. Kobilka, *Nature*, 2009, **459**, 356.

## CHAPTER 20

# *The Possibilities and Limitations of Animal Models for Psychiatric Disorders*

MARK D. TRICKLEBANK\*<sup>a</sup> AND JOSEPH P. GARNER<sup>b</sup>

<sup>a</sup> Lilly Centre for Cognitive Neuroscience, Eli Lilly and Co. Ltd, Erl Wood Manor, Sunninghill Road, Windlesham, Surrey GU20 6PH, UK;

<sup>b</sup> Department of Comparative Medicine and, by courtesy, Department of Psychiatry and Behavioral Sciences, Stanford University, 287 Campus Drive, Stanford, CA 94305-5410, USA

\*Email: tricklebank\_mark@lilly.com

## 20.1 Introduction

Numerous observers have drawn attention to the apparent crisis currently facing the pharmaceutical industry where despite huge financial investment, the number of new chemical entities entering the market has declined year-on-year since the mid 1990s.<sup>1–4</sup> This would, of course, be good news if clinical need had also declined but this is steadfastly not the case. Paradoxically, the range of technologies available to the drug-discovery process has increased massively over the same period such that many thousands of compounds can be rapidly screened and optimized for wanted and unwanted pharmacological activities, pharmacokinetic properties and toxicological effects in a relatively short period of time. The problem lies not with the identification and optimization of molecules interacting with the drug target, but with the validation of the target itself. The issue in a single word is “efficacy” – the average failure rate of NCEs

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

in human trials may be as high as 89%, and 80–90% of these failures are due to lack of efficacy, not (as is widely assumed) due to unexpected toxicity.<sup>2</sup> How can it be that after spending upwards of \$1 billion a compound is finally shown to have no therapeutic benefit in large-scale clinical trials, yet across all therapeutic areas this is now too often the outcome of about 10 years of painstaking work? This chapter concentrates on improving the scientific evidence that has accumulated to support a drug-development programme. It should be noted, however, that whilst there are many issues to confront in the way animal models are used in drug discovery, a recent analysis of failures indicates that in a large number of cases (43%), it was not possible to conclude whether the mechanism had been tested adequately with respect to having clear evidence that the target pharmacology had been functionally engaged.<sup>5</sup>

The industry refers to the loss of NCEs in human trials as “attrition”, and attrition represents the major cost of doing business for most pharma companies because a successful drug has to make back not only the cost of its development, but must also cover the cost of (on average) 10 other failed NCEs developed in the same period of time – indeed none of the major pharma companies can currently break even on their R&D budgets given this burden.<sup>1</sup> The problem raises very serious issues for the companies spending the money, but attrition has far-reaching social and ethical consequences. Companies cannot afford to develop drugs for small patient populations, for “hidden disorders” where patients do not know that help is available, or are unwilling or unable to seek it (even if the disorder itself is extremely common, *e.g.* Trichotillomania,<sup>6,7</sup> or for diseases where socioeconomic or global limitations mean that a drug will not yield the return required (*e.g.* malaria). At the same time, attrition accounts for the vast majority of animal use in drug development (and thus poses significant animal welfare concerns). Reducing attrition therefore would revolutionize the economics of the drug industry, the delivery of public and global health solutions and the welfare of research animals.

The aim of this chapter is to consider how best to improve our design, implementation and analysis of the animal models used to identify potential new medicines for psychiatric disorders. It is not our intention to provide an exhaustive list of such models, nor to review or rank in order of importance or clinical relevance. Instead we wish to highlight what we consider to be some of the key issues that prevent the accurate translation of preclinical neuroscience findings into a clinical setting and how the probability of successful translation can be improved. Our fundamental arguments are that: i) although attrition manifests in translation, it is an issue of the predictive validity of the preclinical animal models; ii) the universality of attrition across drug classes and diseases indicates that it is caused by a systemic set of problems in animal research; iii) the particularly high attrition rates in some drug classes (*e.g.* CNS drugs) indicates particular issues that may be illustrative to the general problem; and iv) that these problems are identifiable, that they are soluble and that we can build animal models with much better predictive validity.

## 20.2 The Drug-discovery Pipeline and an Overview of Potential Issues in Animal Research

These issues are particularly pertinent, but certainly not limited to the search for drugs for the treatment of psychiatric disorders. Psychiatric drug research is, however, currently subject to major disinvestment by many companies who view the financial return to be insufficient to maintain fully active research programmes. The drug-discovery approach has often focused on identifying ligands with high affinity and selectivity for a molecular target (such as a G-protein-coupled receptor or ion channel or enzyme) associated with a neurotransmitter system implicated in the disorders of interest and then screening for supposed beneficial effects of potential compounds in rodent behavioural assays. These assays were developed and validated using the psychopharmacological effects of proven antipsychotic, antidepressant and anxiolytic drugs: any compound demonstrating similar effects was (is) then readily put into the appropriate clinical development bucket (assuming adequate safety and pharmacokinetic parameters), or discarded, because the tests were (are) assumed to have predictive validity. Many interesting drugs have been developed as a consequence, and certainly the tolerability and safety of first-generation psychotherapeutics have been much improved. However, this approach has two fundamental weaknesses. First, it is best suited to finding “more of the same”, and there is no guarantee that a compound acting through a novel mechanism will be identified. Second, it is based on convergent validity (things that look the same), rather than discriminant validity (things that are not different), but discriminant validity is essential for early identification of compounds that will work when taken into human trials. We will return to these issues later in the chapter.

### 20.2.1 Preclinical Explanations for Lack of Clinical Efficacy

Focusing on the role of animal models in attrition, the lack of clinical efficacy in human trials is potentially down to many factors. It may be that:

- (i) Preclinical evidence for the hypothesis is given unrealistic weight in comparison to evidence against the hypothesis, which is either not collected or is ignored.
- (ii) Insufficient attention is paid to experimental design and analysis so that false positives or negatives incorrectly influence judgment.
- (iii) The preclinical data collected to support the hypothesis are irrelevant to the mechanisms underlying the disease of interest.
- (iv) The preclinical data accurately convey the drug responses in a very specific genetically circumscribed population of animals maintained in highly controlled environments, but we fail to discover that this response would not hold true in a heterogeneous mouse or human clinical population (and, therefore, is clinically irrelevant).



- (v) The measurements taken bear at best only face validity to the variables that the experimenter would like to measure and need to be quantified in relation to the disease of interest.
- (vi) The measured effects are confounded by competing responses.
- (vii) And/or there is a real species gap such that the systems being manipulated in experimental animals can never predict outcomes in man.

If point (vii) holds,<sup>8</sup> there is little that can be done. However, while there are major gaps between humans and rodents at all levels of physiology and anatomy, there are still many functional evolutionary links that demonstrate how animal research can inform the study of human anatomy, biochemistry, physiology and behaviour. Indeed, in both mammals and birds, there appear to be universal features of normal psychobiology, such as basic personality dimensions;<sup>9</sup> universal features of normal psychobiology that go awry during the development of disease states, like cognitive bias<sup>10</sup> or executive function;<sup>11,12</sup> and universal epidemiological or neuropsychological features of spontaneously occurring abnormal behaviours in animals and humans,<sup>13–15</sup> – all of which speak to the fundamental utility of animal models.

But what of the other issues? Considering issues (i) and (ii) together, one might imagine that these are the easiest to tackle: at least, they are not dependent on understanding scientific complexity but reflect issues of scientific culture. At the far and rare extreme there is blatant fraud, and scientific work is no more resistant to this than any other aspect of human endeavour; but fraud, clearly, cannot account for even a small proportion of attrition. Science tends to suffer, however, from a bias in the willingness to take positive ( $P < 0.05$ ) and negative ( $P > 0.05$ ) results at face value. Typically a positive result is taken as real; while a negative result more often leads to further experimentation rather than to the judgment that the hypothesis is incorrect. Such further experimentation may be justified loosely “just in case the housing/measures/sample size/animals confounded the result” even though the same confounds could generate a false positive result; and formal methods (such as power tests) that allow assessment of these possibilities without repeating the experiment are rarely applied. This is critical because with each repeated experiment the cumulative risk of a false positive rises; and without replication of positive results there is no way to pick out these false positives. This, issue (i) is, in many ways, a milder empirical dishonesty, which tends to occur in two ways. The first is explicit or complicit publication bias – explicitly, researchers may choose to publish only the study that proves their point, disregarding past failures; and journals are complicit by being less likely to publish negative results. In fact publication bias has an insidious impact on the believability of any given positive result: the assumption that at  $P = 0.05$  we expect roughly 1/20 results to be a false positive is only valid if we also know the proportion of negative results – consider a field where only 1/20 results are positive, but no negative results are published. In this case it is likely that every published result is a false positive. Recent simulation and metaanalyses suggest that such situations may be widespread.<sup>16,17</sup> In fact, negative results can be analyzed and reported just as

empirically as positive results. Of particular use to the field of preclinical research is the “equivalence test”<sup>18,19</sup> because it leads to an ideal experiment where a drug is tested against a negative control (placebo) and a positive control (e.g. a drug known to be effective), and gives a P-value estimate of the likelihood that the drug group is significantly similar to (rather than different from) the control groups. Of course, this assumes effective compounds are available. The positive control acts in such an experiment to give a numerical value to success; so in the absence of a suitable positive control, one could assess a non-significant difference between placebo and drug against a desired detectable effect size (e.g. a 20% improvement in working memory performance).<sup>18</sup>

The second manifestation of issue (i) is the subtle but fundamental logical error of performing “confirmatory” studies, particularly to follow up on an unexpected and *post hoc* result. These results are often great breakthroughs, but this requires us to test their veracity appropriately, including (as mentioned above) replicating a result to rule out the possibility of a false positive. In science we are supposed to test hypotheses by proving ourselves wrong, but confirmatory experiments seduce us into ignoring alternative explanations, instead perpetuating a methodological or interpretational flaw and considering each “confirmation” as an independent piece of evidence (which it isn’t). This is an insidious issue in knockout mouse models. For instance a series of confirmatory experiments appear to suggest that *Hoxb8* deletion in mice is causal to Trichotillomania,<sup>20,21</sup> but these authors never considered the alternative explanation that *Hoxb8* deletion indirectly dysregulates an unrecognized process that is *actually* causal to the behaviour (see for example ref. 22); and they ignore the fact that *Hoxb8* deletion induces a range of phenotypes unseen in human patients (such as complete genotypic penetrance, a lack of female bias and, in initial publications, wide-ranging skeletal abnormalities), or the fact that mutant *Hoxb8* alleles have not been identified in human patients, despite considerable effort to find them. Similarly, aromatase knockout mice appear to show a Trichotillomania phenotype.<sup>23</sup> However, this effect is constrained to mutant male mice (mutant females show no difference from wild-type females) – unfortunately the authors failed to consider the fact that aromatase is essential for male embryos to masculinize the brain and, in fact, the knockout males are merely displaying typical female behaviours for the strain. These examples also illustrate the interconnection of the issues we outline – the errors inherent in these models are also examples of issue (iii) from the perspective of fundamental biology; but they were not caught by the researchers because of logical and interpretational oversights characteristic of issue (i). There is no solution to these kinds of errors other than good science – rigorous application of *falsifiable* testing of alternative explanations and a basic working knowledge of the whole animal biology of the model organism and the clinical physiology and phenomenology in humans.

Issue (ii) is demonstrably a pervasive source of false-positive results. In 2005 *Nature Medicine* reported that statistical auditing of *Nature* journals revealed that 30–40% of published papers contained basic statistical errors.<sup>16</sup> If

anything this problem is intensifying – an analysis of recent papers published in the leading journals of neuroscience revealed that a staggering 50% failed to adopt an appropriate statistical analysis for a factorial design (e.g. when the effect of a drug is compared between knockout and wild-type animals) and made erroneous conclusions as a consequence, with this number rising to 100% of applicable molecular neuroscience papers published in *Nature Neuroscience*.<sup>16,17</sup> While this level of statistical illiteracy is shocking and frustrating to disciplines where statistics is emphasized, it begs the question as to whether it matters. In the case of inappropriate analysis of factorial designs there are clear consequences – factorial designs increase power, reduce sample size and reduce the risk of false positives through multiplicity (repeated testing), but only if appropriately analyzed.<sup>16,24</sup> Similarly, failing to adopt a factorial design in the first place massively elevates false positive rates – for instance, data mining and simulation studies show that a lack of suitable design leads to a ten-fold increase in false discovery rates above what would be expected by chance alone for typical mouse behavioural phenotyping measures.<sup>25,26</sup> Thus it is easy to see how basic errors in study design, analysis and reporting of negative results will lead ultimately to disappointing results in the clinic, yet the benefits of appropriate designs and analyses are well described in textbooks<sup>24</sup> and review articles<sup>19,27</sup> and can be demonstrated with real data.<sup>25,26</sup>

The remaining issues (iii)–(vi) all concern the preclinical assays and disease models that are used to gather the evidence that a new chemical entity (NCE) might have a beneficial role in treating psychiatric disorders – not only how they are designed but how they are implemented and interpreted. As such they are fundamentally interwoven, and we consider them in detail in the remainder of the chapter. However, it is important to distinguish first of all the difference between an assay and a model. An assay is simply a means of quantifying a dependent variable. For instance, measuring the time taken for an animal to press a lever to obtain a food reward following the onset of an auditory or visual stimulus, or counting the number of times the animal presses a lever associated with the delivery of food compared to the number of times it presses a lever not associated with the delivery of food, represent ways of quantifying aspects of behaviour. Embedding these simple measures into rigorously controlled sequences of stimulus, response and reward delivery has allowed the development of assays of attention, vigilance, speed of processing and many other aspects of cognitive, affective and motivational behaviour. However, assays might fail to provide a true picture of the consequences of an experimental treatment (such as a drug) because they do not measure the correct phenomenon (e.g. rodent measures of pain focus on avoidant reflexes or behaviours but, clinically, we want to dull the experience of pain itself, while preserving protective reflexes); the signal-to-noise ratio is too low (i.e. the measure is not sensitive enough, leading to false negatives); the dependent variable is confounded by competing responses (i.e. the measure is overly sensitive and non-specific, leading to false positives); there is a failure to compare and contrast the findings with other related measures (which is an essential quality control to ensure the measure has construct validity, e.g. refs.

28,29); and intra- and inter-laboratory reproducibility of findings is rarely tested (which provides an essential quality control to ensure that the experiment has predictive validity, *e.g.* refs. 25,30–33). Even if these points sound trivial and obvious, constant consideration of *what* a measure is actually measuring is the fundamental intellectual exercise. In contrast the behavioural phenotyping approach that has come to pervade behavioural neuroscience, in adopting a philosophy of “shoot for differences first, ask questions about meaning later”, is defined by the absence of consideration of the true meaning of a measure (either in terms of inherent limitations, or in terms of clinical relevance). Indeed, most behavioural phenotyping measures have been shown to lack formal validity from a traditional behavioural point of view (*e.g.* refs. 34–37), yet the literature applies them nonetheless. Again the issue here is not that screening is inherently a bad idea, but we need to be careful about how it is used – it is a necessary first step especially if the effect of a genetic or pharmacological manipulation is not easy to predict. Screening measures, because they are broad, non-specific and often of weak validity, are inherently far from sufficient and must be followed up with highly specific, well validated measures (just as one would follow up a micro-array screening result with targeted qRT-PCR and protein expression studies).

## 20.3 The Need for Disease Models

Let's suppose we have correctly defined the dependent variable to be quantified and are satisfied by sensitivity, reproducibility and absence of confounds in our measure. We may occasionally be lucky enough for the assay to be able to demonstrate a potential clinical benefit of an NCE in non-manipulated animals. However, typically we rely on the use of a disease model – broadly, an experimental preparation developed in an animal for the purposes of studying a human condition.<sup>38,39</sup> One advantage of a model is that control *versus* model animals may be compared in their response to drug *versus* placebo in a factorial design – ideally only model animals benefit from the drug (as tested by an interaction – exactly the analysis missing from the majority of neuroscience papers.<sup>40</sup> Such a design allows for the detection of non-specific actions of the drug, and/or false positives due to issues with the assay (in either case control animals would be affected too). In addition to this “quality assurance” provided by the control animals treated with the drug, such a design adds both a positive and a negative control (the untreated model and control animals, respectively) – knowing the range between the two is essential for proper analysis of power and interpretation of both positive and negative results.<sup>19</sup>

So far we have discussed “induced models” where a manipulation provides a theoretical description of the way a system or process (or disease) works and allows (over-/under-) expression of a biological variable which an assay quantifies. Induced models are invaluable for basic research, for developing symptomatic treatments and as a tool in experimental design (as illustrated above). However, they are a double-edged sword. First, they are unlikely to reveal unknown early disease mechanisms (for instance, a 6-OHDA lesion

model cannot reveal why Parkinson's patients suffer a loss of dopamine neurones in the first place). Second, they run the inherent risk of translational false positives, in that NCEs discovered in induced models may actually be treating the method of induction of the model, rather than the disease process as it manifests in humans. For instance, in a 6-OHDA lesion model we run the risk that a drug treats the physiology of acute 6-OHDA neurotoxicity, rather than the physiology of gradual progressive dopaminergic cell loss seen in Parkinson's disease (just this difference in timecourse might, for instance, induce very different compensatory responses in the brain). This latter limitation is particularly true for knockout mice – if most human patients do not carry a corresponding loss-of-function allele, we run the risk that the drug will not work in these individuals.

“Spontaneous models”, where the disease develops “naturally” in some animals but not in others, are invaluable for getting around these limitations.<sup>15</sup> Spontaneous models can be difficult to find and validate, but a first clue is often found when a drug proves beneficial in unmanipulated animals. For example, motor impulsivity in ADHD can be quantified using the stop signal reaction time (SSRT) test and is shown to be greater in patients than in age-matched controls.<sup>41</sup> Rats can be similarly trained to withhold a prepotent response to a specific signal and the stop signal reaction time can be measured.<sup>42</sup> Drugs such as modafinil can alleviate the deficit in SSRT in ADHD<sup>43</sup> and it is also able to decrease the SSRT in otherwise untreated rats:<sup>44</sup> in this situation, an animal model of ADHD is not required to demonstrate a significant effect of the drug on SSRT. Consider, though, that without an induced model, this result may be indicative of a false positive – a drug with a general effect that might not be specific to ADHD (even if such a drug might be a useful find for other reasons). Examining individual differences within the animals can provide evidence for a potential spontaneous model (*e.g.* refs. 14,15). For instance, in the study by Eagle *et al.*,<sup>44</sup> the magnitude of the effect of modafinil in rats was much greater in rats with slow reaction times compared to those with faster responding animals: drug effects were more marked in poor performers. Similarly, cholinergic augmentation can alleviate cognitive performance deficits in individuals vulnerable to the effects of sleep deprivation, but has neutral or negative effects on those cognitively resistant to sleep deprivation.<sup>45</sup> There are many experiments where potential clinical benefits are indicated without using a model or experimental perturbation, similar in vain to the atomoxetine studies described above (*e.g.* refs. 41,43,46). However, spontaneous models are best validated (and most useful) when the proportion of the population showing model symptoms can also be manipulated by clinically relevant risk factors (*e.g.* as is seen in barbering as a model of trichotillomania; or stereotypies as a model of stereotypies in autism<sup>14,15,47–50</sup>).

Without such validation, drug effects in unmanipulated animals must be taken with great caution. It does not necessarily follow that drug-induced improvements in performance under these circumstances would necessarily translate into improved performance in, for example, schizophrenia or Alzheimer's disease. It is important to avoid being seduced by the warm feeling

that the drug is capable of influencing those circuits that underlie task performance without undertaking further work to definitively demonstrate that this is the case. For instance, Floresco and Jentsch<sup>51</sup> discussing potential cognitive enhancement argue that: “*if a study is well designed, it uses a sufficiently validated task with appropriately parameterized features and incorporates a sufficiently large number of animals to capture the range of performance, the potential for measuring cognitive enhancement is good*”. However, the abject failure of such compounds to translate into human outcomes in Alzheimer’s Disease (for instance)<sup>52</sup> argues against any certainty in such logic. What if, for instance, the pathological process of the human disease counters the ability of the drug to render improvements in healthy mice or humans? Under these circumstances a disease model – spontaneous or induced – is essential. In either case one would adopt the factorial design illustrated above (for spontaneous models, one would use healthy *versus* diseased animals, *e.g.* ref. 47), and significant effects in control (or healthy) but not in model (or diseased) animals would lead to the key question: how valid is the animal model?

## 20.4 Validation Approaches

Just because a quantity can be measured precisely or repeatedly, this does not mean that it is meaningful. For instance, astrological birth signs or phrenology provide highly accurate and repeatable measures for an individual, but they are not in reality meaningful measures or predictors of personality. This is the essence of the essential distinction between reliability and validity in behavioural theory.<sup>53</sup> While reliability is highly quantifiable, it only tells us the accuracy of a measure. Conversely the validity of a model or assay refers to the degree to which it means what we think it does – does, for instance, the Morris Water Maze really measure deficits in memory equivalent to those seen in Alzheimer’s disease? Validity is an extremely complex concept, and one that cannot be assessed purely quantitatively. Different types of validity represent different questions that challenge the interpretation of the data and are described in Tables 20.1a and 20.1b. Table 20.1a outlines three different “dimensions” of validity widely discussed in the theoretical literature (face/construct/predictive validity; convergent/discriminant validity; and internal/external validity). In principle, questions about the validity of a model or measure can be framed in terms of combinations of these dimensions. Some forms of validity are accessible statistically. Most notably, convergent validity and discriminant validity of a measure are equivalent to the statistical concepts of sensitivity and specificity (and can be assessed as such for a measure using correlational techniques). Other forms of validity require biological or psychological reasoning to build an empirical approach to assessment (*e.g.* construct validity is traditionally assessed using factor analysis<sup>54</sup>). For example, we have recently applied Monte Carlo and experimental techniques to assess the external validity of high-throughput behavioural phenotyping measures.<sup>25,32</sup> However, any deep consideration of validity always involves qualitative biological or psychological reasoning and, in practice, we tend to focus on



**Table 20.1a** Types of experimental validity applied to drug discovery. Validity can be thought of as being comprised of a collection of a number of independent dimensions: face/construct/predictive validity; convergent/discriminant validity; and internal/external validity. A measure or model may excel in one or more combinations of these dimensions. Weak validity in one or more combinations is not inherently fatal, but may limit the appropriate use of the model. For instance a model with construct validity can be used to study underlying pathophysiology even if it lacks predictive validity (*e.g.* mouse models of Alzheimer’s disease<sup>52</sup>) or a knockout mouse model of a rare mutation has internal validity to that patient subpopulation but lacks external validity to the greater patient population.

Validity type	Description
Predictive	The degree to which a test or model predicts future performance but in the current context usually implies the ability of preclinical studies to predict clinical outcome.
Face	The degree of similarity to disease-specific symptoms that are not necessarily produced by the same psychobiological processes.
Construct	The degree to which the molecular and/or structural bases of the disease are reproduced.
-----	
Convergent	When face/construct/predictive aspects of the model or measure are sensitive, and generally similar to the human disease or measure. Confirmatory experiments show convergent validity.
Discriminant	When face/construct/predictive aspects of the model or measure are specific and distinguish the measure or model from competing explanations or alternative human disorders. Experiments attempting self-falsification show discriminant validity.
-----	
Internal	When the measure or model is consistent with the underlying theory of the measure or disease, the biology of the animal and other purported measures of the same property or disease process.
External	When the measure or model is broadly (rather than narrowly) applicable in terms of the disease and the population being modelled.

particular questions reflecting particular combinations of the three dimensions of validity, outlined in Table 20.1b. Despite extensive literature debating the validity of many widely used measures, we are unaware of any group who has tried to validate an animal model of psychiatric disorder systematically applying all of these criteria. But this is not surprising – it is unlikely that any model would be able to fulfil all the criteria outlined. Instead the fundamental exercise in assessing validity is to recognize the limitations of a measure or a model and what it can be used for.

Although in theory a valid measure might be unreliable, reliability is essential for a measure to be practically useful simply because lower reliability requires higher sample sizes to detect the same effect. Thus, in selecting a measure, one



**Table 20.1b** Specific types of experimental validity emphasized in drug discovery. Each of these really reflects specific combination(s) of the dimensions of validity in Table 20.1a.

<i>Validity type</i>	<i>Description</i>
Statistical	When the processes involved are shown to have test-retest and inter-rater reliability; within-lab replicability; and, if relevant, between-lab replicability. Strictly speaking only replicability is relevant to validity, as it speaks to the external validity of a measure or model.
Criterion	The ability of performance in one task to predict performance on another, more ecologically valid test. This is equivalent to internal convergent construct validity. If the gold standard measure is cross-modal from the task ( <i>e.g.</i> if a behavioural test is used to predict a gold-standard hormonal assay), then a case can be made for internal convergent predictive validity.
Clinical	When physiological and behavioural responses in human volunteers or patients mimic those seen in analogous paradigms in animals. This is equivalent to external convergent face validity (which is arguably the weakest form of validity). If the underlying physiology is shown to be homologous, then a claim can be made for external convergent construct validity. If treatment response to efficacious human drugs is shown, then a case can be made for external convergent predictive validity. If non-efficacious compounds in humans are also non-efficacious in the model, then external discriminant predictive validity is demonstrated (which is arguably the most useful validation in terms of preventing false positives in animal models).
Target	When clear beneficial effects of manipulating the target are seen in patients. This is equivalent to external convergent construct validity.

would normally first check for statistical “validity” (Table 20.1b). Reliability is a property of the repeated measurement of individuals (either inter-rater or test-retest reliability). This is somewhat distinct from the issue of replicability – or whether the methodology yields results that are quantitatively variable between experiments. The reproducibility of findings between labs is rarely systematically tested and is insufficiently challenged within labs too, despite extensive evidence that many measures in behavioural neuroscience have unacceptably low reproducibility.<sup>30,31</sup> We have argued that this in fact reflects an issue of validity. Namely that many behavioural neuroscience measures, because they are so influenced by environment at the time of testing, are measuring fleeting states (*e.g.* fear) not stable individual traits (*e.g.* fearfulness<sup>28</sup>). Unfortunately we typically use such measures to ask questions about traits, which is a violation of internal construct validity. Similarly, if we expect a trait to be phenotypically plastic (*i.e.* that animals of the same genotype will behave differently in different environments) then poor replicability can be viewed in part as a failure to recognize that a phenotypically plastic trait should not be constant across different test environments. This is a failure of external validity and is particularly true for many behavioural phenotyping measures. In these circumstances, adopting alternative experimental designs and analyses that recognize these limitations actually both helps to resolve the validity

issues and improves reproducibility.<sup>25,32</sup> This neatly illustrates our point that validity is more about the proper use of a measure than its cut-and-dried suitability. In the remainder of this section, we offer some concrete examples to illustrate these points.

The next most offered approach to validation is outcome prediction: if a clinically proven antipsychotic drug is capable of causing a specific response in an animal, that response becomes a predictor of clinical efficacy. It could be argued that for drug discovery this is the most important validation required: the work is being done in order to (more) accurately predict what will happen in patients. However, reliance on purely convergent predictive validity can easily lead to errors in basic logic, *i.e.* all dogs bark but not all that barks is a dog. Thus, some antipsychotics produce a performance impairment in conditioned avoidance responding whereby an animal fails to respond by moving to a safe compartment to a signal predicting the imminent onset of footshock. This is not merely an extra-pyramidal side-effect (*e.g.* suppression of movement): antipsychotics with limited propensity to induce extra-pyramidal side-effects still prevent shock avoidance, but do not impair the ability to escape the shock itself. All currently available antipsychotic drugs are dopamine D<sub>2</sub> receptor antagonists and there is a strong correlation between D<sub>2</sub> receptor affinity and potency to disrupt conditioned avoidance responding.<sup>55</sup> Is it a leap of faith to rely on convergent validity and propose that drugs devoid of affinity for D<sub>2</sub> receptors yet active in conditioned avoidance testing will be clinically effective antipsychotics? On the one hand it seems that the property of making an animal unable or unwilling to respond to a stimulus predicting an aversive event represents a deficit in performance. On the other hand, it has been argued that the drugs are acting to reduce stimulus salience which, though not optimal for survival in a “normal” individual, might help a schizophrenic cope better with the failure to ignore irrelevant stimuli or the misappropriation of salience to irrelevant stimuli.<sup>56,57</sup> In order to test this hypothesis, non-dopaminergic compounds active in the assay-model (see later) need to be tested as antipsychotics in man – or better, for their ability to reduce the misappropriation of stimulus salience in schizophrenia. Candidate mechanisms include the muscarinic M1/M4 receptor agonist xanomeline<sup>58</sup> and phosphodiesterase PDE10 inhibitors.<sup>59</sup> Interestingly, xanomeline does have some beneficial effects in schizophrenia<sup>60</sup> though its effects on stimulus salience have never been tested in man. The effects of PDE10 inhibitors in schizophrenia are also currently not known.

Face validity on the other hand gives us a warm feeling that there is some meaningful similarity between the responses an animal makes to a given perturbation and the symptoms of the disease of interest. Whilst predictive validity can be measured, theoretically at least, as a success rate, face validity is not easily quantifiable. We are looking for similarity but without knowing the biological substrates of the animal response and the disease face validity is simply the degree of similarity. A good example might be the MAM E17 neurodevelopmental model of schizophrenia,<sup>61,62</sup> which induces in the offspring of mothers treated with the antimitotic agent methylazoxymethanol on embryonic day 17 a pattern of anatomical, histological,

electrophysiological and behavioural effects that bear some similarity to schizophrenia symptoms: there is a great deal of face validity. However, MAM is not an etiological agent and schizophrenia does not arise because of a sudden block of embryonic mitosis over a very short period of time. Does the MAM model therefore have no construct validity despite there being structural similarities to schizophrenia? One might argue that construct validity of the MAM model comes from knowledge that the disease is associated with one or many environmental insults during late embryonic gestation but we currently know so little about how mechanistically this impacts neuronal development, it would be unwise to claim the model held discriminant or external construct validity for the disease. On the other hand, the *Df(16)A<sup>+/-</sup>* transgenic mouse, which models the human genetic micro-deletion on chromosome 22q.11.2 strongly associated with schizophrenia,<sup>63</sup> would be classed as having external construct validity as a disease model, but even here it has to be realized that 22q.11.2 deletion does not make a schizophrenic: there is overlap with autism spectrum and other psychiatric disorders and the deletion only accounts for 1% of familial schizophrenia cases<sup>64-66</sup> (*i.e.* the discriminant validity is unclear). Construct validity is clearly the highest hurdle for any animal model of psychiatric disorder because our knowledge of their molecular basis is so rudimentary. As with the 22q.11.2 micro-deletion, which involves the loss of multiple genes, the hopes for a genetic solution are currently faint, despite diseases like schizophrenia attributing about 80% of the cause to genetic factors.<sup>67</sup> Single-gene knockouts may tell us a little about the function of that gene's products but they won't provide much in the way of a complete disease phenotype against which we can test drugs. More promising would be the addition of a "second hit" approach, as suggested for the complexin2 knockout mouse,<sup>68</sup> where a schizophrenia-like phenotype only becomes evident following a mild parietal neurotrauma applied during puberty.

Let's suppose, however, we have a disease model that seemingly recapitulates some aspects of disease symptomology and that we have a way of quantifying these changes. How else might we test the hypothesized model? One way is to look for criterion validity, *i.e.* the degree to which other methodologies, techniques or experimental paradigms can be used to test the biological significance of the findings. Suppose we see a deficit in a test of working memory measured by an operant delayed-match-to-sample technique. The two-lever operant version of the task has been criticized because the rat may adopt a positional "mediating strategy" to solve the task rather than invoke the prefrontal cortical processes that maintain the original stimuli in memory until a choice can be made. Whilst this may be true, we cannot be certain of the degree to which the strategy adopted has confounded the results. However, if we could demonstrate similar results using a maze or touch-screen-based version of the task where the mediating strategies are either absent or different in nature (criterion validity), or we can use electrophysiological or other methods to indicate the involvement of the frontal cortex in mediating the performance, then we can claim to have provided a degree of convergent validity. To take this one step further, if we are able to demonstrate that task performance is

modulated primarily by factors influencing working memory (*e.g.* delay between sample and choice) but not by factors influencing perception or vigilance (*e.g.* stimulus intensity) or motor function (ability to execute the task), then discriminant validity has been secured.

The last two factors listed in Table 20.1b concern the much hoped for product of preclinical modelling. Firstly, clinical validity is achieved when the results of the preclinical studies can be essentially replicated in man, either in patients or in volunteers. This may or may not be synonymous with target validation which speaks to the achievement of clinical benefit in ways predicted by the preclinical data. Target validation encompasses not only clinical efficacy but also the demonstration of safety and tolerability. Indeed the definition of target validation tends very much to be cut according to the cloth of the company sponsoring the development of the molecule, but here we are only concerning ourselves with efficacy endpoints.

## **20.5 Disease Diagnosis and Animal Models**

Drug discovery programmes focus on clinical need, *i.e.* those aspects of a disease for which the currently available medication has sub-optimal, poor or even no efficacy. In the realm of psychiatric disorders, current efforts are most often focused on the unmet needs of schizophrenia, depression, bipolar disorders, substance abuse, anxiety, attention deficit hyperactivity disorder, obsessive compulsive disorder and, more recently, autism spectrum disorders, where we lack medication for particular aspects of the diseases. For example, there is a general acceptance that the positive symptoms of schizophrenia are well controlled by current antipsychotic drugs but that negative symptoms and cognitive impairment are not. Alternatively, existing medication may be ineffective for a patient subpopulation, as in major depressive disorder where antidepressants are effective in about 60% of cases leaving 40% in a “treatment-resistant” group. Do we have animal models that can aid the discovery and development of treatments for all these disorders with standards of validation as described above? Unfortunately the answer is no. It might then be reasonable to ask how we have managed so far.

Neuropsychiatric disorders are highly complex in origin with multiple genetic factors combining with individual development and environmental experience to generate patterns of behaviour that we ultimately classify as diagnostic entities. In the absence of a clear understanding of the molecular bases of these disorders, rational drug discovery has concentrated on improving the tolerability, pharmacokinetics and toxicology of compounds whose basic mechanism of action was initially discovered serendipitously. Animal models to support such an approach inevitably evolved from psychopharmacological investigations of these initial compounds. In turn, behavioural measures and experimental paradigms were designed to detect in such models the activity of compounds that exert some therapeutic benefit in man. As we have already discussed, this approach limits us to learning what we already know, and hides from us unknown etiological mechanisms, targets and novel therapeutics.

In addition, this approach means that models and disorders are fundamentally different entities. Models often become defined by their drug response; while human diagnostic categories are defined mainly by clinical observation and the patient's self-reporting of symptomatology. This distinction is fundamentally problematic when there is not a one-to-one correspondence between symptom and drug response (*e.g.* SSRIs treat OCD and depression); or between symptoms and etiological mechanism or underlying biology (*e.g.* the same biology may cause different symptoms in different individuals, or the same symptom may have multiple biological causes). Thus, not only do we miss out on novel compounds tied to unknown mechanisms, we also tend to find compounds that treat pharmacological classes of behaviour in the model that may have little relevance to human diagnostic categories or clinical behaviours.

Although neuroscience is now retrospectively trying to fit the biology into the disease, and new models based on biological findings are beginning to emerge, this has been a slow and problematic process. In particular, genetically modified mouse models have been surprisingly ineffective, even when tied to known human biology (*e.g.* ref. 52). We now turn to an alternative tactic for tying human symptomatology to biology that may have particular promise for animal models.

## 20.6 The Symptom Cluster Approach

Despite the inherent “silos” of diagnostic labelling, common risk factors, symptom clusters, physiology, biomarkers and/or endophenotypes can be seen to be dysfunctional across broad diagnostic categories. “*A treatment which reduces impulsive behaviour, for example, might do so whether an individual has a diagnosis of mania, attention deficit hyperactivity disorder or substance abuse, and a treatment for episodic memory problems might prove useful for improving cognition and functional outcome in both mild Alzheimer's disease and first episode schizophrenia.*”<sup>69</sup> This approach recognizes that a single cause and/or single treatment will not be found for any mental health condition and thus obviates the need (and the criticism that follows thereon) for animal models to recapitulate the whole spectrum of a DSM IV diagnosis: such animal models will always be incomplete and unrealistic to achieve. Rather, treatments might be found for specific aspects of several diseases. From our now considerable, if incomplete, knowledge of the biology of affect, motivation, arousal and cognition, an alternative approach to drug discovery might be to examine the neurobiology and pharmacology of the endophenotypes and biomarkers within these broad functional domains *via* a translational neuroscience and experimental medicine approach.

Insel *et al.*<sup>70</sup> have conceptualized the approach within the NIH Research Domains Criteria (RDoC) initiative (<http://mentalhealth.gov/research-funding/rdoc/nimh-research-domain-criteria-rdoc.shtml>). Key to addressing these challenges is a concerted effort to use and expand our knowledge of the biology of the behavioural categories that give rise to clinical need: to understand

the abnormal through investigation of the normal. This is, of course, a reciprocal process – we often come to understand the normal through study of the abnormal. What is important though is putting the abnormal into the framework of our knowledge of anatomy, biochemistry, physiology and behaviour and asking the question as to how genetics and the environment interact to divert the phenotype towards the tail ends of normally distributed characteristics.

A good example of this approach in practice is the European Union Framework 6 project “Imagen” ([www.imagen-europe.com](http://www.imagen-europe.com)), which is investigating mental health and risk-taking behaviour in teenagers *via* a combination of neuropsychological testing, brain imaging and genetic analysis coupled with animal work that examines through translational neuropsychological assays the constructs of impulsivity, inhibition, attention, reinforcement sensitivity and novelty seeking. A clear aim is to examine whether any identified genetic traits in humans influence the animal phenotype following targeted genetic mutation. Any positive associations immediately open the door to dissection of the underlying biology and, potentially, the discovery of valid drug targets.

A similar approach is being taken by another EU-sponsored research programme, the Innovative Medicines Initiative (IMI, [www.imi.europa.com](http://www.imi.europa.com)) NEWMEDS project (Novel Methods leading to New Medications in Depression and Schizophrenia, [www.newmeds-europe.com](http://www.newmeds-europe.com)). As an example, 22q11.2 deletion syndrome has become important for our understanding of the pathophysiology of neurodevelopmental conditions, particularly schizophrenia, which develops in about 20–25% of individuals carrying this CNV. About 1% of schizophrenics have the deletion which is modelled in *Df(16)A<sup>+/-</sup>* mice.<sup>63,64,66</sup> Effort within NEWMEDS is directed to genetic, anatomical and behavioural measures of not only schizophrenia patients but also non-clinical carriers of the CNV, measures that will be similarly taken, as far as is possible, in the *Df(16)A<sup>+/-</sup>* mice. Preliminary evidence suggests that these animals have deficits in spatial memory and a loss of hippocampal-frontal synchrony.<sup>63</sup> By systematically applying tests that are known to engage prefrontal and hippocampal regions in both mice and CNV carriers, the accuracy of the human-to-mouse genetic translation can be assessed: similarities will provide a framework for investigating the pathophysiological processes and symptoms invoked by the manipulation. Some of those symptoms or effects may be relevant to schizophrenia or autism or both. Should the animals or human carriers show no sign of negative affect, disturbed arousal patterns, misattribution of stimulus salience or social interaction, yet have profound disturbances in working memory and executive function, they would provide a means of investigating pathological processes involved in degrading cognitive function in neurodevelopmental disorders and treatments thereof, but would clearly be of no use in the search for treatments for the positive and negative symptoms of schizophrenia.

The importance of a symptom cluster approach is that it forms the basis of a research strategy for personalized medicine (where each patient's unique symptoms are uniquely treated on the basis of their underlying biology). Many



genetic risk factors (such as COMT or Neurexin mutations) are seen across different disease classifications. Especially when such diseases show individual or familial co-morbidity, this implies a biological commonality across diseases, or at least certain symptoms within them. Focusing on the biological dysfunctions responsible for common symptoms may ultimately provide the novel drug targets and compounds that will make up the library of symptom-biomarker-therapeutic indications essential for a successful personalized medicine strategy.

Unfortunately, deep understanding of the neural and pharmacological substrates of most such biomarkers and endophenotypes is missing, even if easier to study than ill-defined diseases. RDoCs gives some guidance on the adoption of the approach and this in modified form is shown in Table 20.2. Clearly, the domains identified are inter-related and interdependent. The need to conceptually separate “assays” from “models” now becomes very apparent. Table 20.2 essentially gives a framework or conceptual infrastructure for the design and use of assays that provide ways of quantifying aspects of cognition, affect, motivation and arousal. Once suitable assays are established, they can be used to investigate the biological processes and, in particular, the brain circuits underlying assay or task performance: animal models of disease are thus replaced by experimental perturbations that can range from temporary pharmacological inactivation of specific brain regions (to define the brain circuits), local injection of pharmacologically specific agents (to define the transmitter systems operating in those circuits) to the over- or under-expression of genetic factors associated with psychiatric symptoms (to achieve understanding of the pathophysiology and to generate a window of opportunity to demonstrate pharmacological modulation in ways that might have positive clinical impact). Many current assays and models fit into a number of the categories. As an example, the forced-swim test, which is often used as an indication of anti-depressant potential, would fit under measures of resilience. However, within the RDoC approach, FST performance would be just one aspect of resilience in

**Table 20.2** The major behavioural domains.

<i>Domain</i>	<i>Description</i>
Cognition	Learning and memory, attention and speed of processing, executive function, social cognition.
Affect	Negative affect: fear (responses to active threat, acute/sustained), anxiety (responses to potential threat, acute/sustained), conflict resolution, frustrative non-reward, negative cognitive bias, anhedonia. Positive affect: responses to reward (acute/sustained), approach motivation, positive cognitive bias, hedonia.
Motivation	Homeostatic drives (food/water, sleep, temperature); effort <i>vs.</i> reward (reward valuation); delay gratification, resilience.
Arousal	Sleep-wake cycle, locomotor activity and accompanying physiological signs (EEG, EMG, CVS, temperature). Resting ( <i>e.g.</i> home cage), activated ( <i>e.g.</i> novel environment) and hyperactivated ( <i>e.g.</i> psychostimulant-induced) states.



particular and affective behaviour in general. The overall affective profile of a molecule would need to be assessed both in non-manipulated animals and in those subjected to clinically relevant experimental perturbations. Vitaly important though, would be an understanding of the circuitry underpinning the behaviour and the pathophysiological relevance of the perturbation.

## **20.7 Use of Biomarkers**

The definition of a biomarker varies according to the context in which the word is used. In the most general of terms it is a biological variable that can be objectively measured and used as an indicator of a normal or abnormal biological process. Biomarkers are most heuristically useful though when contrasted with the concept of an endophenotype. An endophenotype is a heritable and immutable expression of a disease-causing allele that is necessary but not sufficient for the development of the disease.<sup>71,72</sup> For instance, in Phenylketoneuria (PKU), inactivation of phenylalanine hydroxylase is an endophenotype. Combined with dietary availability of phenylalanine, inactive phenylalanine hydroxylase leads to a cascade of developmental changes that ultimately produce the symptoms of the disease. A biomarker is then a necessary step that is measurable in this cascade, which ideally can be manipulated for therapeutic effect. Thus, in PKU we can limit phenylalanine in the diet, which has no effect on the phenylalanine hydroxylase endophenotype, but does reverse the build-up of phenylalanine in the blood (which is the first biomarker in the pathophysiological cascade leading to symptomatic PKU).

Because biomarkers are critical control points in disease development or potential treatment, they are also used as objective measures of target engagement by a drug (as in the use of PET and SPECT for receptor occupancy) as well as being indicators of pharmacological or toxicological responses. Biomarkers may have clinical endpoints that reflect how a patient feels, functions or survives; intermediate endpoints that are statistically correlated with the clinical endpoint or surrogate endpoints that can be used as an appropriate alternative to a clinical endpoint in a clinical trial. How might biomarkers be most usefully used in preclinical research and animal disease modelling? We see two critical roles, first in model development and validation and second in aid of target assessment and refinement.

### **20.7.1 The Role of Biomarkers in Model Development and Validation**

Known biomarkers in humans can be reverse translated – that is adapted for use in animals to give precisely homologous measures of the disease etiology, rather than crude proxies to the disease symptomatology (as is the case for most current behavioural phenotyping measures). Reverse translated biomarkers can then be used in model validation, and basic research (*e.g.* ref. 14). In terms of validating animal models, a model must obviously show biomarkers known to

be necessary to the development of the human disease. An example here would be the measurement of enlarged ventricular size in schizophrenic patients:<sup>73</sup> on the assumption that ventricular size is a valid marker of the disease process, it would be expected that any neurodevelopmental model of schizophrenia ought to lead to increased ventricular size, as is seen in the MAM model described.<sup>61,62,74</sup> Of particular importance, however, is the forward validation of novel biomarkers discovered in animals. Thus, following biomarkers can lead to the discovery of entirely novel disease mechanisms. For instance, in our work with trichotillomania, we developed a reverse translated biomarker-based model.<sup>14</sup> Following the etiology prior to symptom onset led us to discover a new set of biomarkers that predicted later disease,<sup>75</sup> and which suggested an unrecognized early role for oxidative stress, which we confirmed by developing a novel urinary biomarker,<sup>22</sup> and which explains the efficacy of a recently discovered novel compound in humans.<sup>76</sup> Now, prior to significant development of potential targets, we can quickly make a go/no-go decision on the basis of testing for the presence of the novel animal biomarkers in humans. This additional step provides for a significant attrition reduction strategy on the basis of biomarker target confirmation in humans early in the drug-development pipeline, prior to significant development investment.

### **20.7.2 The Role of Biomarkers in Target Assessment and Refinement**

Before determining the functional response to administration of a drug to a whole animal, it is a great advantage to be able to guide dose selection and pre-treatment time on the basis of an objective measurement of engagement with the molecular target. Thus target occupancy, as can be assayed using radio-labelled PET tracers or even with unlabelled tracers when the tracer can be quantified by sensitive mass spectrometry techniques<sup>77,78</sup> while the impact of a range of doses on a later biomarker in the disease process are assessed. Lack of clinical efficacy can be at least partially explained by failure adequately to test the hypothesis *i.e.* by failing to use doses and pre-treatment times that not only occupy the target but also engage that target functionally. The approach can be used to gauge both target validity and specificity. Thus, drug effects should be observed over the dose range that gives rise to significant occupancy of the target. Should effects be present at doses below or above the minimum or maximum occupancy (<0% or >100%, respectively), then it is unlikely that the presumed molecular target of the compound is responsible for all or any of the effects in the etiology assayed by the biomarker.

Currently, intense efforts are underway to identify variables that might be used as biomarkers of the pathological processes underlying psychiatric disorders. The neuropsychological literature provides fertile ground, as distinct symptom classes are known to be specifically correlated with distinct neuropsychological biomarkers in a wide range of disorders, and associated anatomy or physiology is known in man and animals, as exemplified by the work on

Abnormal Repetitive Behaviours.<sup>12,14</sup> Although reverse translated neuropsychological biomarkers can yield success (*e.g.* ref. 12), most neuropsychological paradigms require great skill to carry out and are labour intensive. These measures may not fit well into a traditional behavioural phenotyping screen but they have an invaluable role as a secondary specific screen to allow more educated go/no-go decisions. Brain imaging and electrophysiological technologies are also obvious candidates, particularly when fully integrated with neuropsychological testing. Progress in measuring similar variables in freely moving rats and mice is taking place. For example, oxygen amperometry allows the determination of extra-cellular oxygen concentration in localized brain areas of freely moving rats engaged in behavioural tasks with a temporal resolution similar to that seen with BOLD fMRI.<sup>79,80</sup> Similarly, measuring functional connectivity between brain regions is emerging as an important variable in psychiatric genetics as it provides a neural systems view of genetic risk architecture.<sup>81</sup> Similar approaches in animals are focusing on measuring the synchronization of oscillating electrical activity between brain regions whilst the animals are engaged in spatial memory tasks.<sup>63,82–84</sup> In these examples, we not only have behavioural endpoints but also measures of the circuitry underlying the behaviour.

## 20.8 Conclusions

The current crisis in drug discovery may manifest in human trials, but its core reflects a failure of animal models to predict human outcomes. Animal models are essential to medical research, and can be improved, but doing so first involves understanding why they fail. Here we have discussed and illustrated some of the reasons for these failures. In some cases this is because we are measuring the wrong things in the right model, as is typically the case when we rely on crude phenotyping measures having little relevance to human symptomatology. In some cases it is because the use of crude measures or a lack of knowledge of the human disease or model biology fool us into thinking we have a model when we don't, as is far too often the case with mutant and knockout mouse models. In many cases it is because, like a big-budget movie with a terrible script, despite all the cutting-edge technology the experimental design and statistics are fundamentally lacking. Occasionally, it may be because the human diagnosis does not reflect a coherent or druggable disease physiology. Even if the measure, the statistics and even the model are valid, if we focus on terminal pathophysiology, we fail to model the development of the disease, and end up discovering compounds that treat the way we made the model, not the way the disease truly develops in humans. Thus, the fundamental solution to many if not all of the reasons why models fail is to refocus our efforts on modelling the development of disease, in phenotypically plastic models, based on the known biomarkers of the human disease process. Next-generation models will recapitulate the complex gene-environment interactions of real

human disease development, revealing predictive biomarkers, enabling personalized medicine and revealing novel and effective pharmacologies.

## Acknowledgements

This article was synthesized from extensive discussion over a number of years with colleagues from Eli Lilly and Co. Ltd, the Lilly Centre for Cognitive Neuroscience and colleagues working within the Innovative Medicine Initiative NEWMEDS Project (IMI Joint Undertaking under grant agreement no. 115008), of which resources are composed of EFPIA in-kind contribution and financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013).

## References

1. M. Goodman, *Nat. Rev. Drug Discov.*, 2009, **8**, 927–928.
2. I. Kola and J. Landis, *Nat. Rev. Drug Discov.*, 2004, **3**, 711–715.
3. B. Munos, *Nat. Rev. Drug Discov.*, 2009, **8**, 959–968.
4. S. M. Paul, D. S. Mytelka, C. T. Dunwiddie, C. C. Persinger, B. H. Munos, S. R. Lindborg and A. L. Schacht, *Nat. Rev. Drug Discov.*, 2010, **9**, 203–214.
5. P. Morgan and P. H. Van Der Graaf, *Drug Discovery Today*, 2012, **17**, 419–424.
6. D. W. Woods, C. Flessner, M. E. Franklin, C. T. Wetterneck, M. R. Walther, E. R. Anderson and D. Cardona, *Psychiatr. Clin. North Am.*, 2006, **29**, 487–501, ix.
7. D. W. Woods, C. A. Flessner, M. E. Franklin, N. J. Keuthen, R. D. Goodwin, D. J. Stein and M. R. Walther, *J. Clin. Psychiatry*, 2006, **67**, 1877–1888.
8. D. Premack, *Proc. Natl Acad. Sci. USA*, 2007, **104**, 13861–13867.
9. O. K. van and J. C. Mueller, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 2010, **365**, 3991–4000.
10. E. J. Harding, E. S. Paul and M. Mendl, *Nature*, 2004, **427**, 312.
11. R. Dias, T. W. Robbins and A. C. Roberts, *Nature*, 1996, **380**, 69–72.
12. J. P. Garner, C. M. Thogerson, H. Wurbel, J. D. Murray and J. A. Mench, *Behav. Brain Res.*, 2006, **173**, 53–61.
13. J. P. Garner, C. L. Meehan and J. A. Mench, *Behav. Brain Res.*, 2003, **145**, 125–134.
14. J. P. Garner, C. M. Thogerson, B. D. Dufour, H. Wurbel, J. D. Murray and J. A. Mench, *Behav. Brain Res.*, 2011, **219**, 189–196.
15. J. P. Garner, S. M. Weisker, B. Dufour and J. A. Mench, *Comp. Med.*, 2004, **54**, 216–224.
16. Editorial, *Nat. Med.*, 2005, **11**, 1.
17. J. P. Ioannidis, *PLoS Med.*, 2005, **2**, e124.

18. M. Levine and M. H. H. Ensom, "Post hoc power analysis: An idea whose time has passed?" *Pharmacotherapy*, 2001, **21**(4): 405–409.
19. J. M. Hoenig and D. M. Heisey, *Am. Stat.*, 2001, **55**, 19–24.
20. S. K. Chen, P. Tvrdik, E. Peden, S. Cho, S. Wu, G. Spangrude and M. R. Capecchi, *Cell*, 2010, **141**, 775–785.
21. J. M. Greer and M. R. Capecchi, *Neuron*, 2002, **33**, 23–34.
22. G. Vieira, 45th International Congress of the International Society of Applied Ethology, 2011.
23. R. A. Hill, K. J. McInnes, E. C. Gong, M. E. Jones, E. R. Simpson and W. C. Boon, *Biol. Psychiatry*, 2007, **61**, 359–366.
24. A. Grafen and R. Hails, *Modern Statistics for the Life Sciences*, Oxford University Press, Oxford and New York, 2002.
25. S. H. Richter, J. P. Garner and H. Wurbel, *Nat. Methods*, 2009, **6**, 257–261.
26. S. H. Richter, J. P. Garner, C. Auer, J. Kunert and H. Wurbel, Systematic variation improves reproducibility of animal experiments. *Nat. Methods*, 2010, **7**(3): 167–168.
27. J. A. Newman, J. Bergelson and A. Grafen, *Ecology*, 1997, **78**, 1312–1320.
28. K. A. Miller, *Behaviour*, 2006, **71**, 1323–1334.
29. K. A. Miller, J. P. Garner and J. A. Mench, *Applied Animal Behaviour Science*, 2005, **92**, 113–127.
30. E. J. Chesler, S. G. Wilson, W. R. Lariviere, S. L. Rodriguez-Zas and J. S. Mogil, *Neurosci. Biobehav. Rev.*, 2002, **26**, 907–923.
31. J. C. Crabbe, D. Wahlsten and B. C. Dudek, *Science*, 1999, **284**, 1670–1672.
32. S. H. Richter, J. P. Garner, C. Auer, J. Kunert and H. Wurbel, *Nat. Methods*, 2010, **7**, 167–168.
33. H. Wurbel, *Genes Brain and Behaviour*, 2002, **1**, 3–8.
34. R. Andreatini and L. F. S. Bacellar, *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, 2000, **24**, 549–560.
35. N. Andrews and S. E. File, *Eur. J. Pharmacol.*, 1993, **235**, 109–112.
36. R. E. Brown and A. A. Wong, *Learn. Mem.*, 2007, **14**, 134–144.
37. R. Gerlai and N. S. Clayton, *Trends Neurosci.*, 1999, **22**, 47–51.
38. M. A. Geyer and A. Markou, in *Neuropsychopharmacology: The Fifth Generation of Progress*, ed. K. L. Davis, D. Charney, J. T. Coyle and C. Nemeroff, Lippincott Williams and Wilkins, 2002, pp. 445–455.
39. A. Markou, C. Chiamulera, M. A. Geyer, M. Tricklebank and T. Steckler, *Neuropsychopharmacology*, 2009, **34**, 74–89.
40. S. Nieuwenhuis, B. U. Forstmann and E. J. Wagenmakers, *Nat. Neurosci.*, 2011, **14**, 1105–1107.
41. D. C. Turner, L. Clark, E. Pomarol-Clotet, P. McKenna, T. W. Robbins and B. J. Sahakian, *Neuropsychopharmacology*, 2004, **29**, 1363–1373.
42. D. M. Eagle and T. W. Robbins, *Behav. Neurosci.*, 2003, **117**, 1302–1317.
43. D. C. Turner, L. Clark, J. Dowson, T. W. Robbins and B. J. Sahakian, *Biol. Psychiatry*, 2004, **55**, 1031–1040.
44. D. M. Eagle, M. R. Tufft, H. L. Goodchild and T. W. Robbins, *Psychopharmacology (Berl.)*, 2007, **192**, 193–206.
45. L. Y. M. Chuah and M. W. L. Chee, *J. Neurosci.*, 2008, **28**, 11369–11377.

46. D. C. Turner, T. W. Robbins, L. Clark, A. R. Aron, J. Dowson and B. J. Sahakian, *Psychopharmacology (Berl.)*, 2003, **165**, 260–269.
47. B. D. Dufour, O. Adeola, H. W. Cheng, S. S. Donkin, J. D. Klein, E. A. Pajor and J. P. Garner, *Nutr. Neurosci.*, 2010, **13**, 256–264.
48. J. P. Garner and G. J. Mason, *Behav. Brain Res.*, 2002, **136**, 83–92.
49. C. A. Turner and M. H. Lewis, *Physiol. Behav.*, 2003, **80**, 259–266.
50. C. A. Turner, M. C. Yang and M. H. Lewis, *Brain Res.*, 2002, **938**, 15–21.
51. S. B. Floresco and J. D. Jentsch, *Neuropsychopharmacology*, 2011, **36**, 227–250.
52. K. R. Zahs and K. H. Ashe, *Trends Neurosci.*, 2010, **33**, 381–389.
53. P. Martin and P. Bateson, *Measuring Behaviour: An Introductory Guide*, Cambridge University Press, Cambridge, 1986.
54. J. R. Royce, *Psychological Bulletin*, 2012, **84**, 1098–1106.
55. P. Seeman, *Synapse*, 1987, **1**, 133–152.
56. A. Heinz and F. Schlagenhauf, *Schizophr. Bull.*, 2010, **36**, 472–485.
57. S. Kapur, R. Mizrahi and M. Li, *Schizophr. Res.*, 2005, **79**, 59–68.
58. H. E. Shannon, K. Rasmussen, F. P. Bymaster, J. C. Hart, S. C. Peters, M. D. Swedberg, L. Jeppesen, M. J. Sheardown, P. Sauerberg and A. Fink-Jensen, *Schizophr. Res.*, 2000, **42**, 249–259.
59. S. M. Grauer, V. L. Pulito, R. L. Navarra, M. P. Kelly, C. Kelley, R. Graf, B. Langen, S. Logue, J. Brennan, L. Jiang, E. Charych, U. Egerland, F. Liu, K. L. Marquis, M. Malamas, T. Hage, T. A. Comery and N. J. Brandon, *J. Pharmacol. Exp. Ther.*, 2009, **331**, 574–590.
60. A. Shekhar, W. Z. Potter, J. Lightfoot, J. Lienemann, S. Dube, C. Mallinckrodt, F. P. Bymaster, D. L. McKinzie and C. C. Felder, *Am. J. Psychiatry*, 2008, **165**, 1033–1039.
61. D. J. Lodge and A. A. Grace, *Behav. Brain Res.*, 2009, **204**, 306–312.
62. H. Moore, J. D. Jentsch, M. Ghajarnia, M. A. Geyer and A. A. Grace, *Biol. Psychiatry*, 2006, **60**, 253–264.
63. T. Sigurdsson, K. L. Stark, M. Karayiorgou, J. A. Gogos and J. A. Gordon, *Nature*, 2010, **464**, 763–767.
64. A. S. Bassett, C. R. Marshall, A. C. Lionel, E. W. Chow and S. W. Scherer, *Hum. Mol. Genet.*, 2008, **17**, 4045–4053.
65. A. Brunet, L. Armengol, T. Pelaez, R. Guillamat, V. Valles, E. Gabau, X. Estivill and M. Guitart, *Behav. Brain Funct.*, 2008, **4**, 10.
66. B. Xu, J. L. Roos, S. Levy, E. J. van Rensburg, J. A. Gogos and M. Karayiorgou, *Nat. Genet.*, 2008, **40**, 880–885.
67. N. C. Allen, S. Bagade, M. B. McQueen, J. P. Ioannidis, F. K. Kavvoura, M. J. Khoury, R. E. Tanzi and L. Bertram, *Nat. Genet.*, 2008, **40**, 827–834.
68. K. Radyushkin, A. El-Kordi, S. Boretius, S. Castaneda, A. Ronnenberg, K. Reim, H. Bickeboller, J. Frahm, N. Brose and H. Ehrenreich, *Genes Brain Behav.*, 2010, **9**, 592–602.
69. B. J. Sahakian, G. Malloch and C. Kennard, *Lancet*, 2010, **375**, 1854–1855.
70. T. Insel, B. Cuthbert, M. Garvey, R. Heinssen, D. S. Pine, K. Quinn, C. Sanislow and P. Wang, *Am. J. Psychiatry*, 2010, **167**, 748–751.

71. I. I. Gottesman and T. D. Gould, *Am. J. Psychiatry*, 2003, **160**, 636–645.
72. T. D. Gould and I. I. Gottesman, *Genes Brain Behav.*, 2012, **5**, 113–119.
73. D. G. Owens, E. C. Johnstone, T. J. Crow, C. D. Frith, J. R. Jagoe and L. Kreel, *Psychol. Med.*, 1985, **15**, 27–41.
74. T. M. Sanderson, M. C. Cotel, M. J. O'Neill, M. D. Tricklebank, G. L. Collingridge and E. Sher, *Neuropharmacology*, 2012, **62**, 1349–1358.
75. S. E. Hess, A. C. Lossie, R. L. Meisel, M. Franklin and J. P. Garner. The role of estrogen in the onset and performance of plucking behavior in a mouse model of trichotillomania. *Proceedings of the 38th Annual Meeting of the Society for Neuroscience*, 2008.
76. J. E. Grant, B. L. Odlaug and S. W. Kim, *Arch. Gen. Psychiatry*, 2009, **66**, 756–763.
77. V. N. Barth, E. Chernet, L. J. Martin, A. B. Need, K. S. Rash, M. Morin and L. A. Phebus, *Life Sci.*, 2006, **78**, 3007–3012.
78. E. M. Chernet, L. J. Martin, D. Li, A. B. Need, V. N. Barth, K. S. Rash and L. A. Phebus, *Life Sci.*, 2005, **78**, 340–346.
79. J. Francois, M. W. Conway, J. P. Lowry, M. D. Tricklebank and G. Gilmour, *Neuroimage*, 2012, in press.
80. J. Li, D. S. Bravo, A. L. Upton, G. Gilmour, M. D. Tricklebank, M. Fillenz, C. Martin, J. P. Lowry, D. M. Bannerman and S. B. McHugh, *Eur. J. Neurosci.*, 2011, **34**, 1983–1996.
81. H. Tost, E. Bilak and A. Mayer-Lindenberg, *Neuroimage*, 2011, in press.
82. K. Bechenne, A. Peyrache, M. Kahmasse, P. L. Tierney, Y. Giarney and S. I. Weiner, *Neuron*, 2010, **66**, 936.
83. M. W. Jones and M. A. Wilson, *PLoS Biol.*, 2005, **3**, e402.
84. M. T. Kucewicz, M. D. Tricklebank, R. Bogacz and M. W. Jones, *J. Neurosci.*, 2011, **31**, 15560–15568.



## CHAPTER 21

# *Translational PET Imaging Research in Psychiatry*

RICHARD J. HARGREAVES<sup>\*a</sup> AND  
EUGENII A. RABINER<sup>b</sup>

<sup>a</sup> Merck and Co., WP-42-212, 770, Sumneytown Pike, PO Box 4,  
West Point, PA19486, USA; <sup>b</sup> Imanova Limited, Burlington Danes Building,  
Imperial College London, Hammersmith Hospital, Du Cane Road,  
London, W12 0NN, UK

\*Email: richard\_hargreaves@merck.com

## 21.1 Introduction

While there is certainly no shortage of targets for drug discovery in neuroscience, new safer, and more efficacious medicines remain a considerable unanswered medical need. The heterogeneous nature of most human CNS diseases complicates the elucidation of underlying pathophysiology and can frustrate the evaluation of investigational drugs in the clinic. The same heterogeneity leads to the failure of many novel pharmacological hypotheses to translate to therapeutically useful mechanisms, and most new molecules fail to become successful drugs.

New drug candidates need to differentiate from currently available drugs, and so novel unprecedented mechanisms are often invoked to reach this goal. Yet novel mechanisms carry greater inherent risk of failure as they generally lack clinical validation. Strategies for early decision making are therefore critical to cost-effective drug development, as deferring proof of concept on poorly validated targets to late stage clinical trials is financially unsustainable.

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, www.rsc.org

New drug discovery paradigms are therefore required to enable the efficient evaluation of novel targets to prevent late-stage testing and extensive human exposure of molecules that have no chance of clinical success.<sup>1–3</sup>

The validation of novel CNS targets requires selective small-molecule drug candidates and good lab-to-clinic translation paradigms for early proof of biology and clinical concept studies. CNS biomarkers that can establish target engagement and the pharmacodynamic effects of novel drug candidates are needed to ensure adequate clinical hypothesis testing. If validated CNS biomarkers are absent, and they frequently are, then the clinical evaluation of a novel CNS agent may not inform any further about the promise of the *mechanism* but rather may reach a dead end for the *molecule* and program that may be unjustified.

The broad potential of imaging to optimize CNS drug discovery and development has been reviewed recently.<sup>4,5</sup> In particular, molecular imaging of the brain by positron emission tomography (PET) has become an essential tool in CNS drug development. PET imaging facilitates translation from preclinical species into humans, and the information obtained can be easily interpreted in pharmacological terms, and readily incorporated into the drug development process to set exposure targets and make go/no-go decisions with confidence. This chapter will review the use of PET imaging in CNS drug discovery and in the study of schizophrenia and depression. The technical details of PET as applied to drug development are beyond the scope of this chapter, and interested readers are referred to some recent reviews.<sup>6,7</sup>

## 21.2 Accelerating Proof-of-concept Testing

PET imaging can help validate drug targets in preclinical assays of disease and symptomatology, and focus research on those drug candidates that achieve the highest target engagement or pharmacodynamic effects with the lowest exposures, to maximize therapeutic margins. Linking the degree of target engagement/pharmacodynamics and duration of effect (time on target) to preclinical measures of efficacy (for example, behavioural measures) are critical to molecule selection and hypothesis generation. In early clinical development molecular imaging can be used to link target engagement/pharmacodynamics to drug-induced biological changes that are expected to produce clinical benefit – so-called proof of biology or activity testing. Proof of concept can be declared when target engagement can be linked to a change in a clinically meaningful endpoint. If a drug has adequate target engagement but does not produce the expected biological or clinical effects, the therapeutic concept is flawed and development can be stopped.

To be optimally effective, simultaneous research efforts are required to make sure CNS PET imaging agents reach the clinic at the same time as drug candidates. Biomarkers that come too late have no value as they do not impact decision-making processes. The availability of useful CNS PET ligands allows the development of clinical phase I protocols that weave together first in human single- or multiple-dose safety and tolerability testing with imaging assessment

of target engagement and/or pharmacodynamic effects of potential CNS drugs. This allows early assessment of the relationship between safety/tolerability and target engagement at peak, trough and steady-state plasma drug levels accelerating go/no-go decisions and enabling dose selection for later phase II clinical trials.

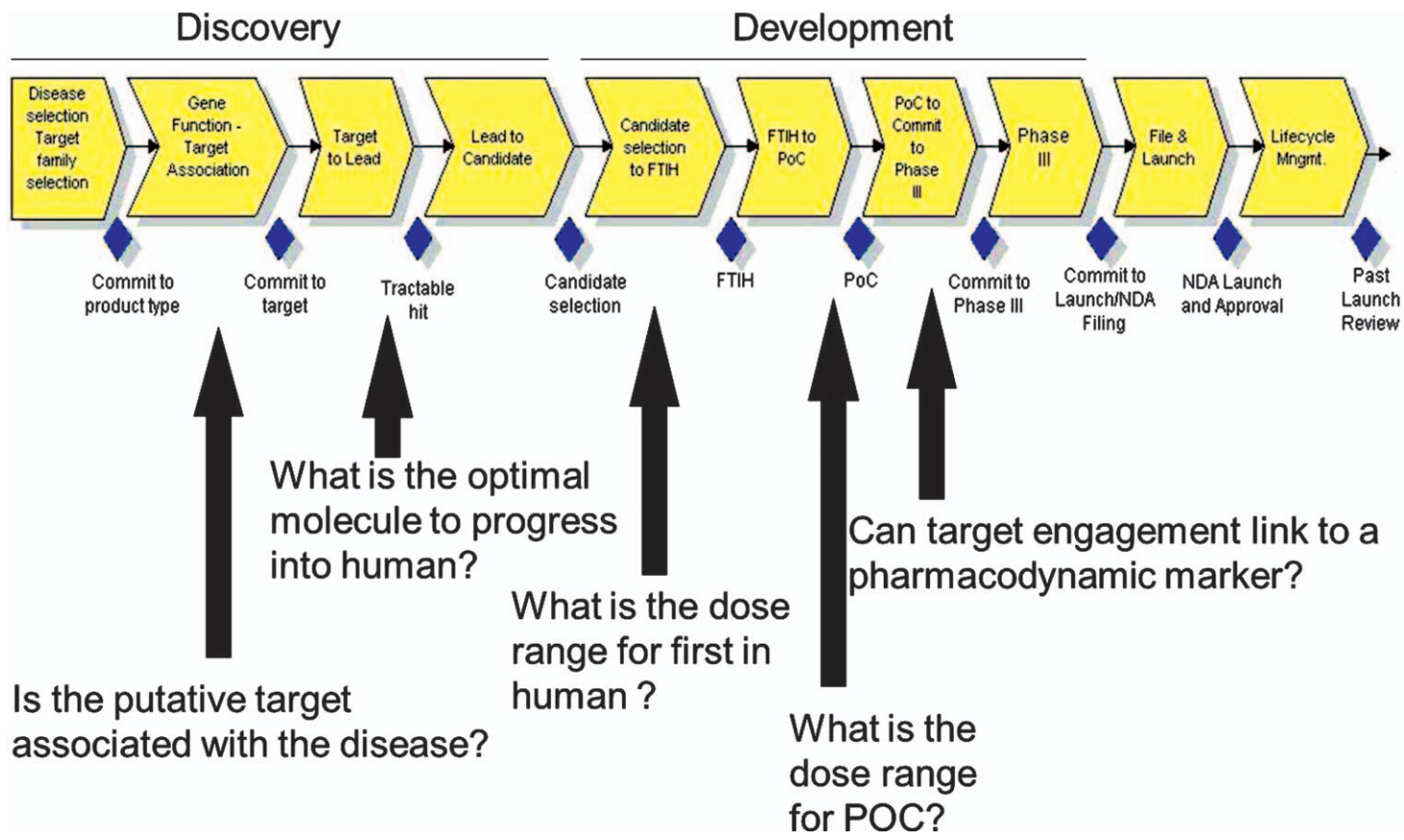
## 21.3 PET Study Designs for CNS Drug Discovery

Molecular imaging in drug development can address three distinct questions: 1) what is the bio-distribution of the novel pharmaceutical in the body, 2) what is the nature of the interaction between the pharmaceutical and its putative target and 3) what are the downstream effects of the interaction of a pharmaceutical with its target. The choice of study to be conducted will depend entirely on the biological questions facing a drug development team, and on the stage of the development program (see Figure 21.1).

### 21.3.1 Biodistribution Imaging

The biodistribution of a drug and investigation of its pharmacokinetics can be examined using compounds labelled with the PET radionuclides  $^{11}\text{C}$  or often  $^{18}\text{F}$ .<sup>8–10</sup> These substitutions do not alter the physicochemical or pharmacological characteristics of the molecule under study as  $^{12}\text{C}$  and  $^{19}\text{F}$  are typically present in drug molecules. Labelling with other radionuclides for the purpose of a biodistribution study should be performed with caution, to ensure the preservation of molecular characteristics in the radio-labelled analogue. The use of tracer amounts of labelled drug allows the utilization of this technique very early in the development phase with limited toxicology testing.

Biodistribution imaging provides information on the anatomical distribution of drugs and their routes of clearance but does not often allow the direct assessment of drug-target interactions. Biodistribution imaging is, however, used in the development of new target engagement PET tracers to establish safety dosimetry (particularly radiation exposure to organs of excretion) for use in drug receptor occupancy studies.<sup>11</sup> In order to be useful for the assessment of target occupancy by drugs, the specific-to-non-specific binding ratio (signal-to-noise ratio; SNR) of the labelled molecule has to be sufficiently high to discriminate between tissues or regions of the brain with high levels of target expression compared to regions lacking the target, in order allow visualization by imaging (typically values  $>1.4$  are required). For the majority of labelled drugs the SNR is too low, and hence direct information about target engagement cannot be derived from the imaging assessments. In the absence of direct target engagement PET tracers, indirect information about target engagement may be obtained by combining PET biodistribution and equilibrium dialysis assays to assess the free brain concentration and BBB transport of CNS drugs.<sup>12,13</sup> A combination of free brain concentration with an estimate of drug affinity (derived from *in vitro* experiments) can provide an estimate of target



**Figure 21.1** Insights from PET imaging that drive decision making in CNS drug development for psychiatric disease.

engagement. However, assumptions about the equivalence of *in vitro* and *in vivo* measured affinity have to be made, and these may differ significantly.

### 21.3.2 PET Target Engagement Imaging

A more direct method of assessing the interaction between drug and target requires the synthesis and development of a target specific radioligand that can be used to quantify target availability. Medicinal chemists in “lock step” with radio-chemists in nuclear medicine now play an increasingly important role in developing radiotracers to determine receptor occupancy by CNS drug candidates. Indeed many successful PET receptor imaging agents have their origin in “rejects” from medicinal chemistry programs designed to produce orally active drugs. The properties of imaging radioligands differ from those of drugs in that they need only be suitable for IV dosing, have short plasma half-lives, have fast brain penetration, have no brain penetrant labelled metabolites and have physicochemical properties that minimize non-specific binding to maximize signal-to-noise sensitivity in addition to high receptor affinity and selectivity. In general the most successful radiotracers have a tissue target concentration/tracer receptor affinity ratio ( $B_{\text{max}}/K_d$ ) of  $\sim 10$  corresponding to an *in vivo* binding potential of  $\sim 1\text{--}3$ . PET radioligands are available for a number of CNS targets (see ref. 5, Table 1, for a representative, though not comprehensive, list) and these continue to be developed as there is now general acceptance of the value of molecular imaging to support cost-effective CNS drug development.

Dedicated molecular imaging groups have been built in companies such as Merck, Pfizer, Novartis, AstraZeneca, Roche, Johnson & Johnson and GlaxoSmithKline, and this has fuelled an increased rate of PET ligand discovery. Over the past 10 years, a significant number of new targets have been made available for PET investigation, including NK1 receptors,<sup>14,15</sup> 5-HT<sub>1B</sub> receptors,<sup>16,17</sup> 5-HT<sub>4</sub> receptors,<sup>18</sup> H<sub>3</sub> receptors,<sup>19,20</sup> CB<sub>1</sub> receptors,<sup>21,22</sup> GlyT1 transporters,<sup>23,24</sup> NPY-Y1<sup>25</sup> and Y5<sup>26</sup> receptors, CGRP receptors,<sup>27</sup> D<sub>2</sub>/D<sub>3</sub> receptors,<sup>28,29</sup> mGluR5<sup>30</sup> and mGluR1<sup>31</sup> receptors. PET neuroreceptor imaging has been most useful for tracking orthosteric antagonist drugs where occupancy has a clear relationship to efficacy but is more difficult to use with agonists, partial agonists and positive and negative allosteric modulators where fractional occupancies may be low and undetectable by PET or binding sites are specific to individual chemical series such that tracers detect both on- and off-target binding. For CNS drugs with multiple targets, PET can be used as an index of target engagement but may not reflect occupancy at all receptor subtypes involved in a clinical response.

It is not, however, always possible to create a radiotracer for all CNS receptor targets. PET has been most successful for GPCRs, enzymes and neurotransmitter transporters but has been relatively unsuccessful in imaging ion channels, other than those formed by inhibitory GABA<sub>A</sub> receptors. The reasons for these failures are diverse but often can relate to target density in the brain, the weak affinity of putative imaging radioligands and access to the

target. Future work is needed to develop the utility of PET to index post-receptor processes, such as changes in second messenger systems or phosphorylation state of activated receptors.

When a PET radioligand is available, estimates of drug occupancy of the target at various doses can be derived by estimating target availability at baseline, and following the administration of the drug. Such studies confirm CNS penetration but, in addition, the measurement of target occupancy at different doses allows the characterization of the plasma-target occupancy relationship and can de-risk the design of larger phase II and III trials by ensuring dose selection is optimized.<sup>32–34</sup> The complexity of target-occupancy study designs has increased significantly from early work, which examined the relationship between drug-dose target occupancy at one time point,<sup>35</sup> to comprehensive evaluation of the timecourse of the relationship between drug plasma concentration and target occupancy.<sup>36</sup> Such drug-target occupancy studies have benefited from the development of efficient experimental designs that employ sequential adaptive designs to minimize the number of PET scans required.<sup>37</sup>

The assessment of the time-dose-occupancy relationship allows the estimation of repeat dose occupancy, from single dose occupancy studies. Estimates of target occupancy in simulated repeat dosing scenario rely on the assumption that the absolute target density does not change between baseline and post-dose assessments and if it does then these estimates may be compromised. Modelling and simulation of repeat-dose occupancy from single-dose experiments allows the conduct of PET occupancy studies early in the drug-development cycle, at the time of first-in-human safety and tolerability studies. The data derived from the PET occupancy studies can be used to refine and reduce the dose range to be tested in repeat-dose safety and tolerability studies, with significant savings in time and resource. However, as repeat dosing of a drug, and indeed disease itself, can lead to up- or down-regulation of the target, it may be more informative to conduct PET occupancy studies at trough plasma levels after repeated dosing to steady state giving consideration to early studies in patients, although even these scenarios will not mimic the effects of chronic long-term drug administration.

### 21.3.3 Pharmacodynamic Imaging

Determination of drug receptor occupancy by PET imaging facilitates clinical proof-of-concept testing but does not reflect brain function. In psychiatry, the brain is about behaviour and behaviour is about functional activity in neuronal circuits. The assessment of “downstream” pharmacodynamic or functional effects of a drug engaging with its target is feasible with PET, though here its utility for drug development is less well established than that for occupancy studies. PET imaging using [<sup>15</sup>O] H<sub>2</sub>O or [<sup>18</sup>F] fluorodeoxyglucose (FDG) has been used to measure brain blood flow and glucose metabolism as indices of neuronal activity and brain function but it has now essentially been supplanted by functional MRI (fMRI), which is a faster, cheaper, safer (due to lack of radiation exposure) and more sensitive way to observe the brain in action.<sup>38,39</sup>



fMRI techniques rapidly measure changes in blood flow or blood oxygenation as surrogates for neuronal activity allowing resting brain activity or network activity evoked deliberately by specific tasks or stimuli to be correlated with neuropharmacology in order to determine where and how drugs may act to produce their therapeutic effects and perhaps also give insight into the etiology of CNS disease itself. The recent advent of combined PET/MRI scanners will provide a huge impetus to the neuroimaging field by enabling PET neuro-receptor imaging of highly selective pharmacological agents to be linked directly in single scanning sessions to brain function with fMRI, brain structure with NMR and brain biochemistry with Magnetic Resonance Spectroscopy (MRS), thereby providing orthogonal yet complementary data to understand the neurobiology of CNS function in health, disease and therapy. A combination of functional MRI with PET occupancy studies will also allow the combination of within-subject pharmacokinetic and pharmacodynamics assessments, and so provide an exciting avenue for future research.<sup>40</sup>

## 21.4 Examples of PET Imaging in CNS Drug Discovery

Translational PET imaging has been used extensively in GABA<sub>A</sub> CNS drug-discovery programs. In the preclinical setting [<sup>11</sup>C] flumazenil PET imaging in baboons was used to show full target engagement of GABA<sub>A</sub> receptors by a novel preclinical non-sedating anxiolytic GABA<sub>A</sub>  $\alpha 2/\alpha 3$  agonist selective drug candidate (TPA-023) at doses that did not produce self-administration or signs of benzodiazepine withdrawal in contrast to a GABA<sub>A</sub>  $\alpha 1/\alpha 2/\alpha 3/\alpha 5$  agonist (TPA-123) and lorazepam.<sup>41</sup> PET imaging was also used to facilitate go/no-go decisions on this class of drugs that lacked  $\alpha 1$  activity by studying MRK-409, an  $\alpha 3$ -subunit preferring GABA<sub>A</sub> agonist.<sup>42</sup> MRK-409 produced anxiolytic-like activity in rodent and primate unconditioned and conditioned models of anxiety with minimum effective doses corresponding to GABA<sub>A</sub> receptor occupancies, depending on the particular model, ranging from ~35% to 65% yet in animals there were minimal overt signs of sedation at occupancies greater than 90%. However, in humans safety and tolerability studies showed that there was pronounced sedation at a dose of 2 mg, which was predicted from animal occupancy data to have low levels of occupancy setting a maximum tolerated dose of 1 mg clinically. Human positron emission tomography studies showed that [<sup>11</sup>C] flumazenil uptake following a single dose of 1 mg MRK-409 was comparable to that of placebo, indicating that occupancy of GABA<sub>A</sub> receptor benzodiazepine binding sites by MRK-409 was below the limits of detection (*i.e.* <10%). Taken together, the data showed that MRK-409 caused sedation in humans at a dose corresponding to levels of occupancy considerably less than those predicted from rodent models to be required for anxiolytic efficacy. Thus, the preclinical non-sedating anxiolytic profile of MRK-409 did not translate into humans and further development of this class of compound was halted.



Similarly,<sup>43</sup> PET was used to help interpret phase I safety and tolerability data for a GABA<sub>A</sub> receptor  $\alpha 5$ -selective inverse agonist, which was shown, unlike non-selective inverse agonists at GABA<sub>A</sub> receptors such as FG7142, to be devoid of anxiogenic effects at receptor occupancies that enhanced cognitive performance in preclinical species.

PET imaging was also used to justify the doses of the selective substance P neurokinin-1 (NK1) receptor antagonists aprepitant and L-759,274 that blocked central NK1 receptors “around the clock” in a series of unsuccessful phase II/III trials of this mechanism in depression<sup>44</sup> and anxiety.<sup>45</sup> These studies, in contrast to preclinical data that suggested potential utility in psychiatric disease, provided strong evidence that abnormalities in the substance P–NK1 receptor neuropeptide signalling system do not underlie the symptoms of depression and anxiety.

## **21.5 PET Imaging in the Treatment and Understanding of Psychiatric Disease**

### **21.5.1 Schizophrenia**

In schizophrenia, PET imaging has shown clear evidence for abnormalities in brain dopaminergic systems<sup>46</sup> and that clinically useful treatments interact with dopamine and serotonin receptors in the CNS.<sup>47</sup>

In the late 1980s Lars Farde and colleagues showed using [<sup>11</sup>C] raclopride that the degree of blockade of the dopamine D<sub>2</sub> receptor by antipsychotic drugs correlated with clinical symptom relief with narrow windows to the degree of blockade that triggered important mechanism-based clinical adverse events such as tardive dyskinesias. These studies enabled informed modification of dosing schedules to open therapeutic windows by optimizing the balance between efficacy and side-effects.<sup>48,49</sup> Interestingly, there appears to be increased occupancy of the dopamine D<sub>2</sub> receptors by dopamine in schizophrenic patients, and the magnitude of increased dopamine stimulation was predictive of treatment response to antipsychotic medication.<sup>50</sup> PET imaging has also been used to show heightened release of dopamine in schizophrenia patients compared to controls and increased striatal dopamine release in patients by stimulants such as methylphenidate and amphetamine associated with activation of psychotic symptoms.<sup>51</sup> Finally, PET using [<sup>18</sup>F] F-DOPA shows increased dopamine synthesis in unmedicated schizophrenic patients, and in subjects with prodromal symptoms of schizophrenia.<sup>52</sup> The PET findings particularly in the associative regions of the striatum and the effectiveness of drugs acting at dopamine D<sub>2</sub> receptors suggest that dopamine dysregulation is central to the disruption of neuronal networks and symptoms in schizophrenia.

PET has also been used to investigate the serotonin system in schizophrenia as atypical antipsychotics with reduced extra-pyramidal symptom (EPS) side-effect liability have activity at 5-HT receptors. The reader is referred to a recent review that summarizes the serotonin PET ligands that have been investigated

to date.<sup>53</sup> Studies measuring the occupancy of therapeutically relevant doses of atypical antipsychotic drugs at D<sub>2</sub> receptors and 5-HT<sub>2</sub> receptors within the same subjects have shown a higher blockade of serotonin 5-HT<sub>2</sub> receptors, compared to dopamine D<sub>2</sub> receptors and the reduced EPS liability compared to typical antipsychotics has been attributed to this activity.<sup>54</sup> The exact relationship between D<sub>2</sub> and 5-HT<sub>2</sub> occupancy and the onset of therapeutic and EPS side-effects, however, appears to vary between atypical agents and this may be a consequence of their activity at other serotonin receptor subtypes such as 5-HT<sub>1A</sub> or the partial agonist nature of their interactions at D<sub>2</sub> receptors. Recent reviews have suggested reanalysis of the profiles of the atypical antipsychotic drug class based on PET imaging findings to facilitate the discovery of the next generation of anti-schizophrenic drugs.<sup>55</sup>

In schizophrenia, the results of 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> PET receptor imaging in patients have been inconsistent and 5-HT transporter binding seems similar to healthy control subjects indicating that serotonin reuptake is not altered in schizophrenia. It is not known whether serotonin release is altered in schizophrenia as, unlike dopamine, radiotracers with sensitivity to 5-HT concentration are just coming into use, and have not yet been applied to clinical populations.<sup>56,57</sup>

Finally, clinical observations that drugs such as phencyclidine and ketamine that block N-Methyl-D-aspartate (NMDA) type glutamate receptors result in transient psychotic symptoms in healthy volunteers and exacerbate psychosis in schizophrenics led to the hypothesis that NMDA hypofunction underlies schizophrenia. The investigation of NMDA receptors in schizophrenia has not yet been possible due to the absence of specific and sensitive radiotracers. NMDA receptor imaging in health and disease remains perhaps one of the most important targets for future PET tracer discovery as it could fuel the discovery of novel therapeutic agents directed to enhancing NMDA receptor function.

### 21.5.2 Depression

PET imaging has been used extensively to understand pharmacotherapy and pathophysiology in depression. PET imaging of the CNS 5-HT transporter (SERT) has consistently shown that occupancies of at least 80% are required for the serotonin reuptake inhibitor drug class to show significant efficacy in the treatment of depression in clinical trials.<sup>58</sup> Lower doses lacked clinical efficacy. This measure is important as it sets an upper occupancy limit for proof-of-concept clinical studies in future drug discovery projects that combine antagonism at SERT with activity at other monoamine receptors such as 5-HT<sub>1A</sub> and inhibition of monoamine transporters such as norepinephrine (NET) and dopamine (DAT) in attempts to gain better and faster onset of efficacy and improved tolerability.

The availability of PET imaging agents for the NET and DAT<sup>59–62</sup> have been developed alongside SERT, which, together with imaging agents for the monoamine catabolic enzymes, monoamine oxidase A and B (MAO-A and

MAO-B), and monoamine receptors (5-HT<sub>1A</sub>, 5-HT<sub>2A</sub> and dopamine D<sub>2</sub>) have helped shape the monoamine hypothesis of depression. Changes in the availability of 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors have consistently been shown in patients with major depression during and after depressive episodes.<sup>63–68</sup> The levels of MAO-A appear elevated, suggesting that reduced endogenous CNS monoaminergic tone in the serotonergic system is a major factor underlying depressive symptomatology.<sup>69</sup>

Recently, PET studies have also shown changes in monoaminergic systems that indicate vulnerability to depressive disorders. Higher MAO-A availability in the prefrontal and cingulate cortex when individuals are at high risk of a major depressive episode, such as during early *postpartum*<sup>70</sup> and in early withdrawal from heavy cigarette smoking,<sup>71</sup> increased SERT binding in winter compared to summer months in brain regions controlling mood,<sup>72–74</sup> persistent decreases in 5-HT<sub>1A</sub> binding after depressive episodes<sup>65</sup> and increased 5-HT<sub>1A</sub> receptor availability after successful psychotherapy.<sup>75</sup>

## 21.6 Challenges to Using Molecular Imaging in Drug Development

The utility of PET imaging is limited practically by the significant cost and the infrastructure required to generate radionuclides and synthesize radiotracers, and limited clinically by radiation exposure and consequent restriction of the number of times that subjects can be studied safely, especially when tracers incorporate high-energy radionuclides such as [<sup>18</sup>F]. The development of novel PET imaging biomarkers can add time and expense to drug-development programs in the early stages, but by eliminating potential drug and mechanism failures early holds the promise of focusing resources on approaches with the highest probability of success in late-phase clinical trials, thereby giving a return on the investment it requires.<sup>76</sup>

Proprietary molecular PET imaging biomarkers are usually linked to specific drug-discovery targets and rarely have broad applicability as disease diagnostics. In contrast, disease imaging biomarkers can generally be considered platform technologies that can characterize patient populations, disease state and response and so have potentially broad cross-target utility with value to diverse therapeutic approaches within a common disease area. In this case, the barrier is that there is little incentive to any one company to bear the considerable cost of clinical qualification and so a different shared solution is required.

Molecular imaging approaches that identify and stratify patients for clinical trials can be used to enrich clinical proof-of-concept studies with patients with defined imaging phenotypes, potentially leading to shorter, smaller and more definitive clinical trials. Stratification using molecular imaging may not only improve the drug development process by defining detection thresholds but ultimately could drive personalized medicine approaches to therapy. There is additionally increasing interest in disease and outcome specific imaging biomarkers that could be used to study disease progression and remission,

potentially serving as surrogate endpoints that could support and speed the registration of new disease-modifying drug therapies.<sup>77</sup>

The validation and qualification of disease-based platform imaging biomarkers that are independent of drug target and mechanism is now increasingly being addressed through formation of large public–private consortia that share the risk and considerable cost of these studies. It is hoped that standardized CNS imaging biomarker measurements that characterize disease progression in patient populations could provide robust entry criteria and baselines for therapeutic trials and so become an important part of the drug application, review and approval process. Examples of these are the Alzheimer’s Disease Neuroimaging Initiative (ADNI: <http://www.adni-info.org/>), Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia (CNTRICS: <http://cntrics.ucdavis.edu/>) and Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS: <http://www.matrics.ucla.edu/>) initiatives.

## 21.7 Summary and Outlook

The translational potential of CNS PET imaging allows the assessment of target occupancy in preclinical species (ideally non-human primates), before first-in-human safety and tolerability studies, to refine dose ranges for exploration in humans. The application of PET in CNS drug development has focused on the determination of brain penetration and target occupancy of novel compounds in phase I. Such information, though expensive to acquire, produces a significant return on investment. PET imaging reduces the overall cost of drug development by eliminating unsuitable molecules at an early stage, defining therapeutic safety windows and selecting dose ranges for testing in late-phase clinical studies. PET imaging has been an important tool for investigating drug–target interactions and the pathophysiology of monoaminergic dysfunction in schizophrenia and depression. The future development of PET/MRI will advance the field by enabling simultaneous imaging of causal pharmacology and functional effects. Public–private consortia will drive future imaging biomarker development for CNS disease and consolidate CNS PET radioligand registries to facilitate sharing of these unique imaging tools to advance investigations of the brain in health disease and therapy.

## References

1. R. Frank and R. Hargreaves, *Nat. Rev. Drug Discovery*, 2003, **2**, 566.
2. R. Hargreaves and J. Wagner, in *In vivo MR Techniques in Drug Discovery and Development*, ed. N. Beckmann, Taylor & Francis, New York, 2006, p. 31.
3. R. J. Hargreaves, *Clin. Pharmacol. Ther.*, 2007, **83**, 349.
4. R. J. Hargreaves, D. Borsook and L. Becerra, *Expert Opin. Drug. Discov.*, 2011, **6**, 597.

5. R. Hargreaves and I. Grachev, in *Imaging in CNS Drug Discovery and Development*, ed. D. Borsook, L. Becerra, E. Bullmore and R. Hargreaves, Springer, 2009, p. 363.
6. T. Jones and E. A. Rabiner, *J. Cereb. Blood Flow Metab.* 2012, advance online publication 21 March 2012: doi: 10.1038/jcbfm.2012.20.
7. P. M. Matthews, E. A. Rabiner, J. Passchier and R. N. Gunn, *Brit. J. Clin. Pharmacol.*, 2011, **73**, 175.
8. D. O. Kiesewetter, E. M. Jagoda, J. E. Starrett Jr, V. K. Gribkoff, P. Hewawasam, N. Srinivas, D. Salazar and W. C. Eckelman, *Nucl. Med. Biol.*, 2002, **29**, 55.
9. M. Bergstrom, A. Grahnén and B. Langstrom, *Eur. J. Clin. Pharmacol.*, 2003, **59**, 357.
10. M. Bergstrom, R. Yates, A. Wall, M. Kagedal, S. Syvanen and B. Langstrom, *J. Pharmacokinet. Pharmacodyn.*, 2006, **33**, 75.
11. D. R. Sprague, F. T. Chin, J.-S. Liow, M. Fujita, H. D. Burns, R. J. Hargreaves, J. B. Stubbs, V. W. Pike, R. B. Innis and P. D. Mozley, *J. Nucl. Med.*, 2007, **48**, 100.
12. R. N. Gunn, C. A. Salinas, K. R. Read, G. Searle, A. D. Ruffo, C. A. Parker, A. J. Stevens, T. Bonasera, P. M. Jeffrey and M. A. Laruelle, *J. Cereb. Blood Flow Metab.*, 2007, **27**, 4–14.
13. R. N. Gunn, S. G. Summerfield, C. A. Salinas, K. R. Read, Q. Guo, G. E. Searle, C. A. Parker, P. Jeffrey and M. Laruelle, *J. Cereb. Blood Flow Metab.*, 2012, **32**, 874.
14. O. Solin, O. Eskola, T. G. Hamill, J. Bergman, P. Lehtikainen, T. Grönroos, S. Forsback, M. Haaparanta, T. Viljanen, C. Ryan, R. Gibson, G. Kieczykowski, J. Hietala, R. Hargreaves and H. D. Burns, *Molecular Imaging and Biology*, 2004, **6**, 376.
15. M. Bergstrom, K. J. Fasth, G. Kilpatrick, P. Ward, K. M. Cable, M. D. Wipperfman, D. R. Sutherland and B. Langstrom, *Neuropharmacology*, 2000, **39**, 664.
16. J. D. Gallezot, N. Nabulsi, A. Neumeister, B. Planeta-Wilson, W. A. Williams, T. Singhal, S. Kim, R. P. Maguire, T. McCarthy, J. J. Frost, Y. Huang, Y. S. Ding and R. E. Carson, *J. Cereb. Blood Flow Metab.*, 2010, **30**, 196.
17. J. D. Andersson, M. E. Pierson, S. J. Finnema, B. Gulyas, R. Heys, C. S. Elmore, L. Farde and C. Halldin, *Nucl. Med. Biol.*, 2011, **38**, 261.
18. L. Marner, N. Gillings, R. A. Comley, W. F. C. Baare, E. A. Rabiner, A. A. Wilson, S. Houle, S. G. Hasselbalch, C. Svarer, R. N. Gunn, M. Laruelle and G. M. Knudsen, *J. Nucl. Med.*, 2009, **50**, 900.
19. T. Hamill, N. Sato, M. Jitsuoka, S. Tokita, S. Sanabria, W. Eng, C. Ryan, S. Krause, N. Takenaga, S. Pate, Z. Zeng, D. Williams, C. Sur, R. Hargreaves and H. Burns, *Synapse*, 2009, **63**, 1122.
20. S. Ashworth, E. A. Rabiner, R. N. Gunn, C. Plisson, A. A. Wilson, R. A. Comley, R. Y. K. Lai, A. D. Gee, M. Laruelle and V. J. Cunningham, *J. Nucl. Med.*, 2010, **51**, 1021.

21. H. D. Burns, K. van Laere, S. Sanabria-Bohorquez, T. Hamill, G. Bormans, W. Eng, R. Gibson, C. Ryan, B. Conolly, S. Patel, S. Krause, A. Vanko, A. van Hecken, P. Dupont, I. de Lepeleire, P. Rothenburg, A. Stoch, J. Cote, W. Hagmann, J. Jewell, L. Lin, P. Liu, M. Goulet, K. Gottesdiener, J. Wagner, J. de Hoon, L. Mortelmans, T. Fong and R. Hargreaves, *Proc. Natl Acad. Sci. USA*, 2007, **104**, 9800.
22. G. E. Terry, J. Hirvonen, J.-S. Liow, S. S. Zoghbi, R. Gladding, J. T. Tauscher, J. M. Schaus, L. Phebus, C. C. Felder, C. L. Morse, S. R. Donohue, V. W. Pike, C. Halldin and R. B. Innis, *J. Nucl. Med.*, 2010, **51**, 112.
23. J. Passchier, G. Gentile, R. Porter, H. Herdon, C. Salinas, S. Jakobsen, H. Audrain, M. Laruelle and R. N. Gunn, *Synapse*, 2010, **64**, 542.
24. T. G. Hamill, W. Eng, A. Jennings, R. Lewis, S. Thomas, S. Wood, L. Street, D. Wisnoski, S. Wolkenberg, C. Lindsley, S. M. Sanabria-Bohorquez, S. Patel, K. Riffel, C. Ryan, J. Cook, C. Sur, H. D. Burns and R. Hargreaves, *Synapse*, 2011, **65**, 261.
25. E. D. Hostetler, S. M. Sanabria-Bohorquez, Z. Zeng, L. T. Gantert, M. Williams, S. S. O'Malley, P. J. Miller, H. Fan, M. Kameda, M. Ando, S. Ozaki, S. Tokita, H. Ohta, D. Williams, C. Sur, J. J. Cook, H. D. Burns and R. J. Hargreaves, *NeuroImage*, 2011, **54**, 2635.
26. N. Erondur, I. Gantz, B. Musser, S. Suryawanshi, M. Mallick, C. Addy, J. Cote, G. Bray, K. Fujioka, H. Bays, P. Hollander, S. Sanabria-Bohorquez, W. Eng, B. Langstrom, R. J. Hargreaves, H. D. Burns, A. Kanatani, T. Fukami, D. J. MacNeil, K. M. Gottesdiener, J. M. Amatruda and K. D. Kaufman, *Cell Metab.*, 2006, **4**, 275.
27. S. Sanabria, H. Fan, S. O'Malley, P. Miller, Z. Zeng, C. Ryan, K. Riffel, M. Williams, W. Eng, S. N. Gallicchio, I. Bell, H. Selnick, C. Salvatore, R. Hargreaves, S. Kane, S. Graham, C. Sur, J. J. Cook and E. Hostetler, World Molecular Imaging Congress, Sept 23–26, 2009, Montreal, Canada.
28. P. Shotbolt, A. C. Tziortzi, G. E. Searle, A. Colasanti, J. van der Aart, S. Abanades, C. Plisson, S. R. Miller, M. Huiban, J. D. Beaver, R. N. Gunn, M. Laruelle and E. A. Rabiner, *J. Cereb. Blood Flow Metabol.*, 2012, **32**, 127.
29. J.-D. Gallezot, J. D. Beaver, R. N. Gunn, N. Nabulshi, D. Weinzimmer, T. Singhal, M. Silfstein, K. Fowles, Y.-S. Ding, Y. Huang, M. Laruelle, R. E. Carson and E. A. Rabiner, *Synapse*, 2012, in press.
30. T. G. Hamill, S. Krause, C. Ryan, C. Bonnefous, S. Govek, T. J. Seiders, N. D. P. Cosford, J. Roppe, T. Kamenecka, S. Patel, R. E. Gibson, S. Sanabria, K. Riffel, W. Eng, C. King, X. Yang, M. D. Green, S. S. O'Malley, R. Hargreaves and H. D. Burns, *Synapse*, 2005, **56**, 205.
31. E. Hostetler, W. Eng, A. Joshi, S. Sanabria-Bohorquez, H. Kawamoto, S. Ito, S. S. O'Malley, S. M. Krause, C. Ryan, S. Patel, M. Williams, K. A. Riffel, G. Suzuki, S. Ozaki, H. Ohta, J. J. Cook, H. D. Burns and R. J. Hargreaves, *Synapse*, 2010, **65**, 125.
32. M. Bergstrom, R. J. Hargreaves, H. D. Burns, M. R. Goldberg, D. Sciberras, S. A. Reines, K. J. Petty, M. Ogren, G. Antoni, B. Langstrom, O. Eskola, M. Scheinin, O. Solin, A. Majumdar, M. Constanzer, W. P.



- Battisti, T. E. Bradstreet, C. Gargano and J. Hietala, *Biol. Psychiatry*, 2004, **55**, 1007.
33. R. J. Hargreaves, *J. Clin. Psychiatry*, 2002, **63**(1), 18.
34. G. Searle, J. D. Beaver, R. A. Comley, M. Bani, A. Tziortzi, M. Slifstein, M. Mugnaini, C. Griffante, A. A. Wilson, E. Merlo-Pich, S. Houle, R. Gunn, E. A. Rabiner and M. Laruelle, *Biol. Psychiatry*, 2010, **6**, 392.
35. C. J. Bench, A. A. Lammertsma, R. J. Dolan, P. M. Grasby, S. J. Warrington, K. Gunn, M. Cuddigan, D. J. Turton, S. Osman and R. S. J. Frackowiak, *Psychopharmacology*, 1993, **112**, 308.
36. S. Abanades, J. van der Aart, J. A. R. Barletta, C. Marzano, G. E. Searle, C. A. Salinas, J. J. Ahmad, R. R. Reiley, S. Pampols-Maso, S. Zamuner, V. J. Cunningham, E. A. Rabiner, M. A. Laruelle and R. N. Gunn, *J. Cereb. Blood Flow Metab.*, 2011, **31**, 944.
37. S. Zamuner, R. Gomeni and A. Bye, *Nucl. Med. Biol.*, 2002, **29**, 115.
38. D. Borsook, L. Becerra and R. J. Hargreaves, *Nat. Rev. Drug Discovery*, 2006, **5**, 411.
39. R. J. Hargreaves, L. Becerra and D. Borsook, in *Biomedical Imaging: The Chemistry of Labels, Probes and Contrast Agents*, ed. M. Braddock, The Royal Society of Chemistry, Cambridge, UK, 2011, ch. 8, p. 411.
40. E. A. Rabiner, J. Beaver, A. Makwana, G. Searle, C. Long, P. J. Nathan, R. D. Newbould, J. Howard, S. R. Miller, M. A. Bush, S. Hill, R. Reiley, J. Passchier, R. N. Gunn, P. M. Matthews and E. T. Bullmore, *Mol. Psychiatry*, 2011, **16**, 826.
41. N. A. Ator, J. R. Attack, R. J. Hargreaves, H. D. Burns and G. R. Dawson, *J. Pharm. Exp. Ther.*, 2010, **332**, 4.
42. J. R. Attack, K. A. Wafford, L. J. Street, G. R. Dawson, S. J. Tye, N. G. B. Agrawal, K. van Laere, G. Bormans, S. M. Sanabria-Bohorquez, I. De Lepeleire, J. De Hoon, A. van Hecken, H. D. Burns, R. M. McKernan, M. G. Murphy and R. J. Hargreaves, *J. Psychopharmacology*, 2010, **25**, 314.
43. W. Eng, J. R. Attack, M. Bergstrom, S. Sanabria, L. Appel, G. R. Dawson, D. Sciberras, R. J. Hargreaves, B. Langstrom and H. D. Burns, *Neuropharmacology*, 2010, **59**, 635.
44. M. Keller, S. Montgomery, W. A. Ball, M. F. Morrison, D. B. Snavely, G. F. Liu, R. J. Hargreaves, J. Heitala, C. R. Lines, K. L. Beebe and S. A. Reines, *Biol. Psychiatry*, 2006, **59**, 216.
45. D. Michelson, R. J. Hargreaves, R. C. Alexander, T. P. Ceesay, J. Hietala, C. R. Lines and S. A. Reines, *Int. J. Neuropsychopharmacol.*, 2012, **Mar 20**, 1–11. [Epub ahead of print] PubMed PMID: 22433185.
46. O. D. Howes, A. Egerton, V. Allan, P. McGuire, P. Stokes and S. Kapur, *Curr. Pharm. De.*, 2009, **15**, 2550.
47. O. D. Howes, P. Fusar-Poli, M. Bloomfield, S. Selvaraj and P. McGuire, *Curr. Pharm. Des.*, 2012, **18**, 459.
48. L. Farde, F. A. Wiesel, C. Halldin and G. Sedvall, *Arch. Gen. Psychiatry*, 1988, **45**, 71.



49. L. Farde, R. J. Mack, S. Nyberg and C. Halldin, *International Clinical Psychopharmacology*, 1997, **12**, S3.
50. A. Abi-Dargham, J. Rodenhiser, D. Printz, Y. Zea-Ponce, R. Gil, L. S. Kegeles, R. Weiss, T. B. Cooper, J. J. Mann, R. L. van Heertum, J. Gorman and M. Laruelle, *Proc. Natl Acad. Sci. USA*, 2000, **97**, 8104.
51. A. Abi-Dargham, R. Gil, J. Krystal, R. M. Baldwin, J. Siebyl, M. Bowers, C. H. van Dyck, D. Charney, R. B. Innis and M. Laruelle, *Am. J. Psychiatry*, 1998, **155**, 761.
52. O. D. Howes, A. J. Montgomery, M. C. Asselin, R. M. Murray, I. Valli, P. Tabraham, E. Bramon-Bosch, L. Valmaggia, L. Johns, M. Broome, P. K. McGuire and P. M. Grasby, *Arch. Gen. Psychiatry*, 2009, **66**, 13.
53. L. M. Patterson, B. R. Kornum, D. J. Nutt, V. W. Pike and G. M. Knudsen, *Med. Res. Rev.*, 2011, published online in Wiley Online Library (wileyonlinelibrary.com): doi 10.1002/med.20245.
54. D. Mamo, S. Kapur, C. M. Shammi, C. Papatheodorou, S. Mann, F. Therrien and G. Remington, *Am. J. Psychiatry*, 2004, **161**, 818.
55. G. G. Grunder, H. Hippus and A. Carlsson, *Nat. Rev. Drug Discovery*, 2009, **8**, 197.
56. S. J. Finnema, A. Varrone, T. J. Hwang, B. Gulyas, M. E. Pierson, C. Halldin and L. Farde, *Synapse*, 2010, **64**, 573.
57. K. Ridler, C. Plisson, E. A. Rabiner, R. N. Gunn, B. Easwaramoorthy, A. Abi-Dargham, M. Laruelle and M. Silfstein, *Synapse*, 2011, **65**, 1119.
58. J. H. Meyer, A. A. Wilson, S. Sagrati, D. Hussey, A. Carella, W. Z. Potter, N. Ginovart, E. P. Spencer, A. Cheok and S. Houle, *Am. J. Psychiatry*, 2004, **161**, 826.
59. C. Halldin, L. Farde, C. Lundkvist, N. Goniovert, Y. Nakasima, P. Karlsson and C.-G. Swahn, *Synapse*, 1996, **22**, 386.
60. A. Varrone and C. Halldin, *J. Nucl. Med.*, 2010, **51**, 1331.
61. Y.-S. Ding, K.-S. Lin, V. Garza, P. Carter, D. Alexoff, J. Logan, C. Shea, Y. Xu and P. King, *Synapse*, 2003, **50**, 345.
62. Y.-S. Ding, K.-S. Lin, J. Logan, H. Benveniste and P. Carter, *J. Neurochem.*, 2005, **94**, 337.
63. W. C. Drevets, E. Frank, J. C. Price, D. J. Kupfer, D. Holt, P. J. Greer, Y. Huang, C. Gautier and C. Mathis, *Biol. Psychiatry*, 1999, **46**, 1375.
64. P. A. Sargent, K. H. Kjaer, C. J. Bench, E. A. Rabiner, C. Messa, J. Meyer, R. N. Gunn, P. M. Grasby and P. J. Cowan, *Arch. Gen. Psychiatry*, 2000, **57**, 174.
65. J. H. Meyer, S. McMain, S. H. Kennedy, L. Korman, G. M. Brown, J. N. DaSilva, A. A. Wilson, T. Blak, R. Eynan-Harvey, V. S. Goulding, S. Houle and P. Links, *Am. J. Psychiatry*, 2003, **160**, 90.
66. Z. Bhagwagar, E. A. Rabiner, P. A. Sargent, P. M. Grasby and P. J. Cowen, *Mol. Psychiatry*, 2004, **9**, 386.
67. Z. Bhagwagar, R. Hinz, M. Taylor, S. Fancy, P. Cowen and P. Grasby, *Am. J. Psychiatry*, 2006, **163**, 1580.
68. J. H. Meyer, *Semin. Nucl Med.*, 2008, **38**, 287.

69. J. H. Meyer, A. A. Wilson, S. Sagrati, L. Miler, P. Rusjan, P. M. Bloofield, M. Clark, J. Sacher, A. N. Voineskos and S. Houle, *Arch. Gen. Psychiatry*, 2009, **66**, 1304.
70. J. Sacher, A. A. Wilson, S. Houle, P. Rusjan, S. Hassan, P. M. Bloomfield, D. E. Stewart and J. H. Meyer, *Arch. Gen. Psychiatry*, 2010, **67**, 468.
71. I. Bacher, S. Houle, X. Xu, L. Zawertailo, A. Soliman, A. A. Wilson, P. Selby, T. P. George, J. Sacher, L. Miler, S. J. Kish, P. Rusjan and J. H. Meyer, *Arch. Gen. Psychiatry*, 2011, **68**, 817.
72. N. Praschak-Rieder, M. Willeit, A. A. Wilson, S. Houle and J. H. Meyer, *Arch. Gen. Psychiatry*, 2008, **65**, 1072.
73. H. G. Ruhe, J. Booij, J. B. Reitsma and A. H. Schene, *Eur. J. Nucl. Med. Mol. Imaging*, 2009, **36**, 841.
74. J. Kalbitzer, D. Erritzoe, K. K. Holst, F. A. Nielsen, L. Marner, S. Lehel, T. Arentzen, T. L. Jernigan and G. M. Knudsen, *Biol. Psychiatry*, 2010, **67**, 1033.
75. H. Karlsson, J. Hirvone, J. Kajander, J. Markkula, H. Rasi-Hakala, J. K. Salminen, K. Nagren, S. Aalto and J. Hietala, *Psychol. Med.*, 2010, **40**, 523.
76. D. Borsook, L. Becerra, E. Bullmore and R. Hargreaves, in *Imaging in CNS Drug Discovery and Development*, ed. D. Borsook, L. Becerra, E. Bullmore and R. Hargreaves, Springer, 2009, p. 3.
77. D. Borsook, L. Becerra, E. Bullmore and R. Hargreaves, in *Imaging in CNS Drug Discovery and Development*, ed. D. Borsook, L. Becerra, E. Bullmore and R. Hargreaves, Springer, 2009, p. 381.

## CHAPTER 22

# *Future Drug Discovery for Psychiatric Disorders*

DARRYLE D. SCHOEPP\* AND RICHARD  
J. HARGREAVES

Merck Research Laboratories, Merck and Company, Inc.,  
1351 Sumneytown Pike, UG/4CDS013, North Wales, PA 19454, USA

\*Email: darryle\_schoepp@merck.com

In the past decades, tremendous strides have been made in the diagnosis and treatment of psychiatric diseases. The neuroscience area has a proven track record of delivering some of the most medically important and commercially successful medicines across all therapeutic areas. New drugs were introduced that include safer and/or more effective therapies for schizophrenia, depression and anxiety disorders. It is now becoming increasingly clear that these psychiatric disorders are co-morbid with many neurological diseases such as chronic pain and neurodegenerative disorders like Alzheimer's disease, Parkinson's disease and multiple sclerosis such that the boundaries between psychiatry and neurology are becoming progressively blurred increasing the need for innovative medicines.

Current scientific, business and regulatory challenges in the pharmaceutical industries make it imperative to prioritize and focus research and development. This means focusing on a reduced number of therapeutic areas, diseases and new disease targets emphasizing those with the greatest potential for clinical and commercial success. In this context, central nervous system (CNS)

---

RSC Drug Discovery Series No. 28

Drug Discovery for Psychiatric Disorders

Edited by Zoran Rankovic, Matilda Bingham, Eric J. Nestler and Richard Hargreaves

© The Royal Society of Chemistry 2012

Published by the Royal Society of Chemistry, [www.rsc.org](http://www.rsc.org)

research and development is being reduced in a number of companies, despite a vast increase in knowledge that has occurred in the field of neuroscience and the tremendous unmet medical need for new medicines. Many drugs for psychiatric diseases were initially discovered by serendipity without real knowledge of their mechanism of actions; subsequently newer and safer drugs were discovered after it was known what mechanisms to target. Success rates for the development of new drugs for psychiatric disease and indeed CNS diseases in general are today considered some of the lowest of all therapeutic area categories in the industry. There is the, possibly correct, perception that the knowledge of CNS disease pathophysiology relative to other areas is only in its infancy, making it harder to find new drug targets that have a high probability that they will work. It is important, however, to remember that the term “CNS disease” encompasses a constellation of disorders and symptoms in the fields of psychiatry and neurology, and that the maturity of the science and therapy varies widely across them. As a consequence future success in CNS drug discovery will also vary, and choosing which areas to focus upon becomes increasingly important to have a chance of getting a return on research investments and meeting the unmet medical needs of patients. Current therapies offer some help but new, safer and more efficacious therapies are still needed. In order to be worthy of investment, new drug candidates need to differentiate from currently available drugs. This often means that a novel mechanism is needed in order to reach this goal yet novel mechanisms carry greater inherent risk of failure.

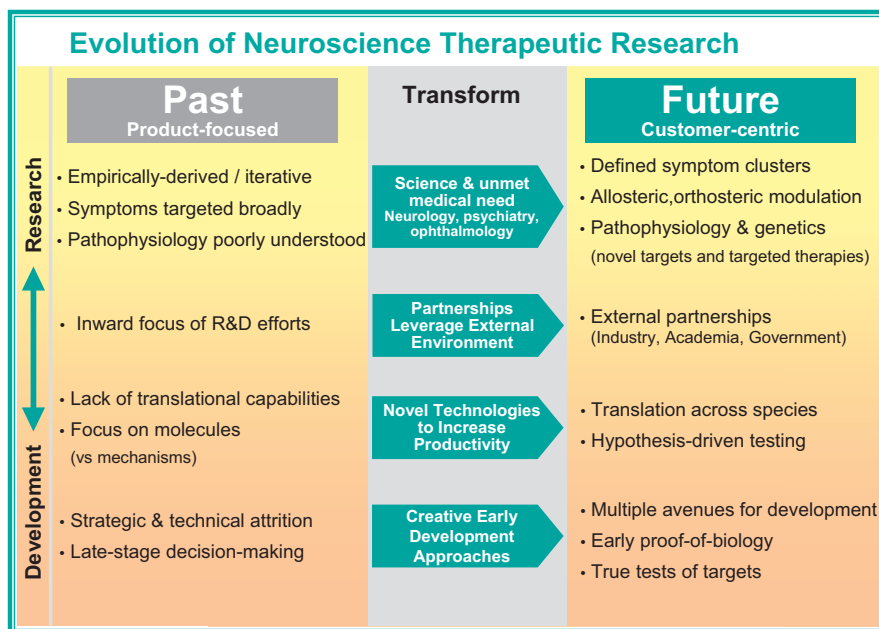
We now have many new and truly revolutionary technologies to study brain function and pharmacology, a detailed knowledge of receptor pharmacology that followed the cloning of virtually all known neurotransmitter receptor families and a much greater and still evolving understanding of disease genetics systems neurobiology and pathophysiology. Most targets we work on today are, however, still unprecedented and so are considered novel and lacking clinical validation; it is perhaps then not surprising that the probability of success in gaining positive clinical outcomes is lower compared to the past where iterations of known mechanisms were being pursued. Compounding this reality is the fact that many of the current preclinical models for CNS diseases were developed and validated with the existing drugs, and may have unknown value for predicting the success of novel mechanisms. Whilst *in vivo* animal assays provide important tests of central pharmacology, they do not generally replicate the complexity and heterogeneous nature of either human psychiatric illness or the treatment-resistant populations that are in most need of a new therapy.

This brings up the question of what we have learned from the past that can be applied to the future. How can we now operate differently in a riskier, cost-constrained environment to increase the likelihood that we will choose the best mechanisms and chemically synthesize the best molecules to invent effective new medicines for psychiatric diseases?

Neuroscience drug discovery is a multi-disciplinary effort in which medicinal chemistry plays a central role. Medicinal chemists can now

readily develop small molecule probes that have good selectivity and activity against the many potential CNS therapeutic targets. The brain remains a privileged compartment in the body and medicinal chemists have developed the art and science of designing molecules that readily diffuse into the brain and have resistance to efflux transporters, yet possess the correct physicochemical characteristics to be orally absorbed. Validation of prospective CNS targets is critically dependent on the development of well-behaved small-molecule probes. Indeed, tool molecules that have near drug-like selectivity and activity against the target are often required. Unlike peripheral targets, the use of antibodies or other modalities for target validation in the brain is less tractable, so small molecules remain key to this mission. The better the tool molecule and the more we understand if it is performing as desired in humans, then the more certain we are that the target hypothesis is being tested. Also, the better the molecule the higher the chance it might become the drug itself if the mechanism is proven to work. All too often sub-optimal molecules fail to test hypotheses and so confuse and complicate paths for future development of new drug entities, especially in neuropsychiatric disease. It is important to remember that in early development the goal is always to test the mechanism and not the molecule and so taking time to ensure that de-risked high-quality drug candidate molecules with good therapeutic margins to safety and toxicity are developed before advancing in clinical development is critically important. New technologies have revolutionized how we do our work and some promising new approaches and drug targets have now been validated in human studies. For each novel mechanism and target a tool box of target engagement (PET imaging) and pharmacodynamic markers (fluid and functional such as EEG and fMRI) needs be built to ensure that the best molecules and doses are used in clinical proof-of-concept studies designed to test therapeutic hypotheses and provide true target validation.

Medicinal chemists together with radio-chemists in nuclear medicine now play an increasingly important role in developing PET tracers to establish brain penetration and target engagement that can guide interpretation of preclinical experiments and help select doses for clinical trial. The design, in parallel with drug candidate synthesis, of precursor molecules suitable for rapid labelling at high specific activity with  $^{11}\text{C}$  and  $^{18}\text{F}$  radionuclides to produce imaging radioligands that have high receptor affinity, target selectivity, fast brain penetration and physicochemical properties that minimize non-specific binding to maximize signal-to-noise sensitivity is now an important feature of many discovery programs. Indeed the lack of a “bio-marker tool box” or critical measures of drug target engagement and pharmacodynamics to guide dose selection probably explains why so many targets from the 1990s are still out there, still being pursued and have not yet been shown to work or put to rest. It is a sobering thought that many different companies may keep failing on the same targets for the same reason and do not know it for the lack of biomarkers to guide development and go/no-go decision making.



**Figure 22.1** A new framework for neuroscience drug discovery.

Despite these challenges, the field of neuroscience is progressing more rapidly than ever before. A new framework for neuroscience drug discovery is emerging (Figure 22.1). Genetic information and a better understanding of pathophysiology are driving our selection of new targets. We have to continue to drive better understanding of CNS diseases, create and implement enabling technologies and focus our efforts on the most important scientific questions and mechanisms (not molecules) as the path to future success in the CNS area. Finding more ways to get this done in precompetitive space using collaborations and consortia seems like a worthwhile goal. This could start with a handful of important targets that have a reasonable chance of success based on what is known about the hypothesis, distribution and density of expression across preclinical and human species, knowledge of human genetics, human pathophysiology, human pharmacology and further supported by pharmacology in animal experiments that include human transgenic models and surrogate pharmacology.

The challenge for neuroscientists and medicinal chemists working on CNS disorders has never been greater. Working closely together with clinical colleagues in drug development and patient care, they have the opportunity to address some of the most personally devastating and costly illnesses society faces today. The low-hanging fruit may have been picked, but the initial discoveries – many made decades ago – still leave significant room for improvement. The diversity of neuropsychiatric disease provides opportunities for

meaningful advances in treatment that could address high unmet needs for control of symptoms in schizophrenia, depression, bipolar illness, anxiety, sleep disorders, drug addiction and autism. Learning from the past, exploiting the scientific advances in understanding the function of the brain in health and disease, together with implementation of a new, more objective biomarker-driven framework for CNS drug discovery will hopefully provide a firm foundation for future drug discovery and the impetus for true innovation in neuropsychiatric drug therapy.



# *Subject Index*

References to figures are given in *italic type*. References to tables are given in **bold type**.

- A-68930, 135, *135*
- A-77636, 135, *135*
- A-86929, *135*, 136
- ABBF, *127*, 128
- ABS-103, 353
- ABT-239, *142*, 143
- ABT-436, 309
- ABT-925, 44
- acetylcholine, 125, *125*
- acetylcholine receptors
  - muscarinic
    - allosteric agonists, 139–40
    - positive allosteric modulators, 137–9, *139*
  - nicotinic, agonists, 124–9
    - allosteric modulators, 129–32
- acetylcholinesterase inhibitors, 517
- acid dissociation constant, 483–5, 495–6
- ACP-103, 43
- addiction *see* substance-related disorders
- affective disturbances, 15
- agomelatine, 211–12, *211*
- agoraphobia, 228
- ALB-127128, 313–14
- alcohol abuse, 360–1
  - glutamate dysregulation, 367–8
  - neuropeptide substance P, 362–3
  - opioid system, 370
- alcohol/aldehyde dehydrogenases (ADH/ALDH), 375
- almorexant, 427–9, 428, 434
- Alzheimer's disease, 73, 208
- AM-251, *373*
- amiloride-sensitive cation channel 1 (ACCN1), 239
- amitriptyline, 259, *260*, **345**
- AMPA, 262
- AMPA receptors, 101–2
  - role in fear response, 233–4
- AMPA receptor modulators, 69–71, 73, 266–7
- discovery case study, 107–9
  - clinical trial, 119
  - crystallographic assays, 107–9, 114–16
  - development candidate
    - characterization, 116–18
    - hit to lead optimization, 109–14
  - discovery landscape, 103–4
  - preclinical studies, 71–2
  - screening, 104–7
- amphetamine, 359, 359
- amygdala, 244
  - fear conditioning and, 229–31, 230, 237
- amyotrophic lateral sclerosis (ALS), 263
- Angelman syndrome, 388
- anhedonia, 171
- animal models
  - anxiety disorder, 163–6, 229
  - central nervous system
    - penetration, 453–5, 460–1

- animal models (*continued*)
  - depression, 163–6
  - disease diagnosis and, 547–8
  - induced, 540–1
  - need for, 540–2
  - relationship between human and animal physiology, 537
  - spontaneous, 541
  - stress, 166–7
  - symptom cluster
    - approach, 548–51
    - use of biomarkers, 551–3
    - validation approaches, 542–7
- aniracetam, 70, 70, 106
- antidepressants, 8
  - catecholamines, 162–3
  - cytokines, 174–5
  - epigenetic, 175
  - ketamine, 174
  - monoamine oxidase inhibitors (MAOI), 160–1, 345–6
  - multiple target drugs, 523–7
  - sedating, 6
  - serotonin (ant)agonists, 205–12
  - SSRI *see* selective serotonin reuptake inhibitors
  - treatment of substance addiction, 373–4
- antipsychotics, 4, 18–19, 35–6
- AMPA receptor modulators
  - see* AMPA receptor modulators
- atypical, 18–19, 36–9
- catechol-*O*-methyl transferase inhibitors (COMT), 144
- D-serine, 65–9
- DAAO inhibitors, 66–9
- dopaminergic receptor (ant)agonists, 43–7
  - D<sub>1</sub>, 132–7
- GABA<sub>A</sub> agonists, 143–4
- glycine transporter
  - inhibitors, 58–60
  - GlyT1, 60–6
- histamine H3 receptor
  - antagonists, 142–3
- metabotropic glutamate receptor
  - modulators, 73–8
- multiple ligand, 48–51, 519–20
- receptor affinity, 37–9, **38**
- serotonin receptor (ant)agonists
  - 5-HT<sub>1A</sub> partial agonists, 39–40
  - 5-HT<sub>2A</sub> antagonists, 42–3
  - 5-HT<sub>2C</sub> agonists, 40–2
- treatment of autistic spectrum disorders, 397
- antisauvagine, 283
- anxiety disorders, 5–6, 159–60, 226–7
  - animal models, 163–6
  - CRF1 receptor antagonists, 280–3
  - drug targets
    - glutamate and GABA, 244–5
    - neuropeptides, 245
  - epidemiology, 227–8
  - fear conditioning, amygdala, 229–31
  - see also* panic disorder; post-traumatic stress disorder
- aprepitant, 288
- arginine vasopressin (AVP), 276, 283–4, 307–10, 321
- aripiprazole, 37, 37, **38**, 39, 39–40, 48, 193, **194**, 360, 361, 397
- AS057278, 68–9
- ASC-1, 65–6
- asenapine, 39
- association studies, schizophrenia, 30–1
- astressin-2B, 283
- ATC0065, 312
- atomoxetine, 7, 108, 259, 260
- attention deficit-hyperactivity disorder (ADHD), 6–7, 104
- attrition, 535
  - causes of lack of clinical efficacy, 536–40
- ATV0175, 312
- autism, 7, 384–5
  - diagnosis, 385–6, **386**

- autism spectrum disorders (ASD), 385
  - aetiology, 387–8
    - chromosomal rearrangement, 388–90
    - copy number variations, 390–1
    - environmental factors, 396
    - mutations, 391–6
  - biomarkers, 404–5
  - clinical outcome measures, 402–4
  - epidemiology, 386–7
  - oxytocin, 401
  - treatments
    - current, 396–7
    - GABA<sub>B</sub> agonists, 400
- AVE-5997, 44
- azabicyclo-based D<sub>3</sub> antagonists, 46, 46
- benzodiazepines, 6
- benzolactams, 51
- benzothiadiazines, 70–1
- benzyl quinoline carboxylic acids, 138
- BF-2649, 142
- bifeprunox, 39–40, 40, 48
- BIIE-0246, 317, 318
- BINA, 76, 77
- biodistribution imaging, 560–1
- biomarkers, 551
  - role in model development, 551–3
- bipolar disorder, 6, 8, 258–9
  - causes, 334–7
  - classification, 333–4
  - diagnosis, 338–9
  - omega-3 fatty acids, 351
  - protein kinase C signalling cascade, 348
  - symptoms, 337–8
  - treatment, 339–40
    - carbamazepine, 342
    - lamotrigine, 342–3
    - lithium, 340–1
    - neurontin, 352–3
    - new agents, 346–7
    - noradrenergic and specific serotonergic antidepressants, 344–5
    - off-label, 352–3
    - protein kinase C inhibitors, 349–51
    - selective serotonin reuptake inhibitors (SSRI), 343–4
    - serotonin-norepinephrine reuptake inhibitors, 344
    - topamax, 353
    - valproate, 341–2
- Bleuler, Eugen, 14
- blood-brain barrier (BBB), 446–7, 466–7
  - P-glycoprotein efflux, 486–8
  - transporters, 447
- blood-CSF barrier (BCSFB), 447–8, 466
- BMS-562086, 303, 306
- BP-897, 44
- brain homogenate, 451
- brain imaging *see* neuroimaging studies
- brain slices, 451
- brain to total plasma ratio (B/P), 451–2, 452, 469
- brain-derived neurotrophic factor (BDNF), 169
- bromocriptine, 132, 133
- bupropion, 260, 373, 373
- buspirone, 190, 205
- cannabinoids, 372–3
- carbamazepine, 340, 342, 342
- catechol-*O*-methyl transferase inhibitors (COMT), 144
- catecholamines, 162–3
- cationic amphiphilic drugs (CAD), 500
- CBIO (6-chlorobenzo[d]isoxazol-3-ol), 68, 68
- CDPPB (3-cyano-*N*-(1, 3-diphenyl-1*H*-pyrazol-5-yl)benzamide), 80, 81, 267, 268
- ceftriaxone, 264, 265, 365

- central nervous system (CNS)  
 penetration, 445–6  
 assessment  
   *in silico*, 449  
   *in vitro*, 449–51  
   *in vivo*, 451–5  
   microdialysis, 455  
   receptor occupancy, 455–6  
   serial CSF approach, 454–5,  
     456–8  
 hERG, 491–3  
   discrete structural  
     modifications, 496–8  
 ligand efficiency, 502–3  
 optimization strategies, 459–60,  
   **473–4**  
 P-glycoprotein, 486–9  
 partition coefficient control, 493–5  
 pharmacokinetic methods, 468–72  
 physicochemical properties, 472–4  
   binding affinities, 483–5  
   hydrogen bonding  
     potential, 476, 478–80  
   lipophilicity, 475–8  
   molecular weight, 482  
   polar surface area, 480–1  
   rotational bond count, 485  
 predictive validity of animal  
   models, 460–1  
 relationship between fluid  
   compartments, 447–8  
 cerebral blood flow, panic  
   disorder, 236  
 cerebrospinal fluid, 447, 466  
   factors impacting, 458–9  
   sampling, 448  
   *see also* blood-cerebrospinal fluid  
     barrier  
 Cerep BioPrint, 490  
 CGS-21680, 424  
 chlorpromazine, 18, 35, 36  
 circumventricular organs (CVO), 234  
 citalopram, 185, 187, 210, 344, 501  
 Clinical Antipsychotic Trials of  
   Intervention Effectiveness  
   (CATIE), 57  
 clinical studies  
   AMPA receptor  
     modulators, 72–3, **105**, 119  
     causes of lack of efficacy, 536–40  
     limitations, 534–5  
 clomipramine, **345**  
 clozapine, 36–7, 39–40, 501  
 CNQX (6-cyano-7-nitroquinoxaline-2,  
   3-dione), 365  
 cocaine, 358–9, 361–2, 369, 371,  
   431–2  
 codeine, 375  
 cognitive impairment, 100, 123–4  
 computed tomography (CT), 26  
 confirmatory studies, 538  
 copy number variations  
   (CNV), 390–1  
 CORT-108297, 284, 286  
 corticosteroids, 285  
 corticotrophin-releasing factor  
   (CRF), 172, 276–7, 302–6, 321,  
   425  
   CRF1 antagonists, 280–3, 301–6  
   *see also* CRF-binding protein  
 cortisol, 278, 285  
 Cowden syndrome, 393–4  
 CP-101606, 264, 266  
 CP-154526, 303, 304  
 CP-316311, 301, 303, 304, 305–6  
 CP-376395, 303, 306  
 CP-810123, 128–9, 128  
 CRF *see* corticotrophin-releasing  
   factor  
 CRF-binding protein (CRF-BP),  
   283  
 Cushing's syndrome, 285  
 CX-516, 70, 71–2, 73, 104, 106, 264  
 CX-546, 70, 71  
 CX-691, **105**  
 CX-717, 73, 104, **105**  
 cycloserine, 233  
 cyclothiazide, 70, 106, 264  
 cyclothymia, 334  
 cytochrome P450 (CYP)  
   enzymes, 490–1  
 cytokines, 174–5

- D-amino acid oxidase (DAAO), 66–9  
depression, 4–5, 159–60  
    aetiology  
        catecholamine hypothesis, 162  
        serotonin, 161  
    animal models, 163–6  
    epidemiology, 184–5  
    neuropathology, 167–8  
        hypothalamus, 168–9  
        nucleus accumbens, 171  
        prefrontal cortex, 170–1  
    orexin and, 433  
    PET imaging, 566–7  
designed multiple ligands  
    (DML), **512**  
    AChE/SERT, 517–18  
    activity optimization, 516–20  
    antidepressant, 523–7  
    antipsychotic, 527–30  
    molecular weight, 521–2  
    screening approaches, 514–15  
    screening vs. knowledge-based  
        approaches, 515–16  
desimpramine, 259, 260  
dexamethasone (DEX), 240, 284–5,  
    284, 286–7  
diagnosis, 2–3  
    animal models and, 547–8  
    symptom cluster approach, 548–9  
Diagnostic and Statistical Manual for  
    Mental Disorders (DSM-IV), 2, **3**  
    anxiety disorders, 227  
    autism, 385–6  
dihydroxidine, 135–6, 135  
dinapsoline, 135, 135  
discrete structural modification  
    (DSM), 496–8  
dizocilpine, 264  
DNA methyl transferases, 175  
dopamine, 132, 132  
    receptor (ant)agonists  
        D<sub>1</sub> agonists, 132–7  
        D<sub>2</sub>, 20, 37–8, 49–51  
        D<sub>3</sub>, 44–7  
        D<sub>4</sub>, 47–8  
        D<sub>5</sub>, 133  
        multiple ligand, 520, 527–9  
        substance abuse, 359–62  
        reuptake inhibition, 202–6  
        schizophrenia and, 20–2  
        transporters, 358–60  
DORA-22, 434–5  
dorsomedial hypothalamus  
    (DMH), 234  
DOV-21947, 199, 200, 517  
doxapram, 234  
doxepin, **345**  
drug metabolism pharmacokinetics  
    (DMPK), 468–72  
dual orexin receptor antagonist  
    (DORA-22), 434–5, 435  
duloxetine, 197, 259, 260  
  
EAAT2 activator, 264  
Elavil *see* amitriptyline  
electrophysiology, 27–8  
EMD-281014, 43, 43  
endoxifen, 349, 349, 350  
Enerzer *see* isocarboxazid  
entacapone, 144, 144  
ephexin 5, 394  
equivalence test, 538  
escitalopram, 260  
eszopiclone, 434–5  
excitatory amino acid transporters  
    (EAAT), 261  
ezlopitant, 363  
  
Farampator, 104, **105**  
fear conditioning, 229  
fenfluramine, 490, 490–1  
fenoldopam, 134  
fexofenadine, 499  
fight-or-flight response, 233  
fixed dose combination  
    (FDC), 511–12  
FKBP5, 290–1  
flashbacks, 228–9  
flesinoxan, 206  
fluoxetine, 185, 197, 210, 344  
fluvoxamine, 185, 344  
forced swim test, 166

- fragile X syndrome (FXS), 392–3, 398
  - GABA<sub>B</sub> agonists, 400
  - mGluR negative allosteric modulators, 398–400
  - STX-209, 400–1
- free drug hypothesis, 468
- G-protein coupled receptors (GPCR), 280
- GABA, 19
  - GABA<sub>A</sub> selective agonists, 143–4
  - GABA<sub>B</sub> selective agonists, 400
  - role in panic disorder, 234, 238, 244
  - role in schizophrenia, 23–4
  - role in sleep disorders, 434
- galanin, 290, 320–1
- $\gamma$ -aminobutyric acids *see* GABA
- GBR-12909, 360
- genetic studies, 2
  - autism spectrum disorders, 388–96
  - bipolar disorders, 335
  - narcolepsy, 418–20
  - post-traumatic stress disorder (PTSD), 243–4
  - schizophrenia, 28–9, 30–1
- genome-wide association studies (GWAS), 238–9
- gepirone, 189, 190, 206
- geriatric populations, 196–7
- glucocorticoid receptors (GR), 286–7
- glutamate carboxypeptidase II, 103
- glutamate receptors, 267–8
  - group I, 78–81
  - group II, 74–7, 268
  - metabotropic *see* metabotropic glutamate receptors
  - release inhibitors, 263–4
  - role in anxiety disorders, 244, 244–5, 267–8
  - role in autistic spectrum disorders, 398, 398–400
  - role in mood disorders, 260–1, 261, 262
  - role in schizophrenia, 102–3
  - role in substance-related disorders, 363–9
  - transporter enhancers, 264–5
  - see also* NMDA receptors
- glycine transporter inhibitors, 58–60
- GlyT1, 59, 64
  - amino acid based, 60–2
  - non-amino acid based, 62–4
- GlyT2, 59
- GR-205171, 288, 289
- GSK-172981, 477, 478
- GSK-189254, 142, 143
- GSK-256471, 477, 478
- GSK-282254, 312, 313
- GSK-561679, 281, 282
- GSK-729327, 105
- GTS-21, 125
- GW-803430, 312
- GW-856464, 312, 313
- GW-876008, 281, 282, 303, 305
- haloperidol, 18, 35–6, 48, 501
- hERG, 491–3
  - carboxylic group incorporation, 498–9
  - partition coefficient control, 493–5
- heritability, 2
- heroin, 359, 369
  - opioid system, 371–2
  - replacement therapies, 373
- high-throughput screening (HTS), 514–15
- hippocampus, 169–70
- histamine receptor
  - antagonists, 142–3, 142
- hydrogen bonding potential, 476, 478–80
- 5-hydroxyindole, 130
- 5-hydroxytryptamine *see* serotonin
- hypocretin receptor 2 gene (HCRTR1), 239
- hypocretins, 433
- hypomania, 338
- hypothalamus, 168–9

- hypothalamus-pituitary-adrenal (HPA) system, 276–7, 277  
FKBP5, 290–1  
neuropeptide receptors, 302–6  
  neuropeptide Y, 317–19  
non-mood related effects, 279–80  
role in anxiety disorders, 240  
role in mood disorders, 278–9
- iloperidone, 37, 37, **38**, 39  
imipramine, 197, **345**, 501  
imprinting centres, 388  
indalpine, 185  
Innovative Medicines Initiative, 549  
insomnia, 6  
interleukins, 174  
International Classification of Diseases (ICD-10), 2–3  
interstitial fluid (ISF), 447  
intra-cellular fluid, 447  
ispapirone, 189
- JNJ-1930942, 130, 131–2  
JNJ-2765074, 318, 318  
JNJ-5207787, 318, 319  
JNJ-10397049, 430–1, 430
- K-41498, 283  
Kanner, Leo, 384  
 $\kappa$  opioid receptors, 314–16, 370  
ketamine, 173–4, 173, 264  
knockout studies, central nervous system exposure, 453
- L-687414, 63  
L-701324, 365  
lamotrigine, 260, 263, 342–3  
learned helplessness, 164  
lecozotan, 209, 209  
Leonhard, Karl, 14  
life events, 336–7  
ligand efficiency, 482, 502–3  
lipophilicity, 475–8  
lithium, 259, 340–1, 348  
litoxetine, 195  
lobelane, 360, 361–2  
*Lobelia inflata*, 361  
long-term potentiation (LTP), 72, 169  
Lu-AA-33810, 318, 318  
Lu-AE-58054, 141, 141  
lurasidone, 49  
LY-341495, 268  
LY-354740, 75, 75, 234, 365  
LY-379268, 365  
LY-392098, 264  
LY-404039, 75, 75  
LY-404187, 264  
LY-451395, **105**, 106  
LY-686017, 288  
LY-2140023, 75–6, 75  
LY-4581395, 104
- M-100907, 43  
mAChR *see* muscarinic acetylcholine receptors  
magnetic resonance imaging (MRI), 26  
  functional, 232, 236–7  
  prefrontal cortex, 232  
major depressive disorder (MDD), 258, 259  
mania, 337–8, 349–51  
maximal electroshock threshold test (MEST), 114  
mazindol, 199–200  
MCL-0042, 320  
MCL-0129, 320  
MDMA, 359  
MDR-1 *see* P-glycoprotein  
Measurement and Treatment  
  Research to Improve Cognition in Schizophrenia (MATRICS), 15, 124, **124**, 137  
melanin-concentrating hormone (MCH) receptors, 311–14, 493  
melanocortins, 319  
melatonin, 336  
memantine, 264  
mesial temporal lobe, 22  
metabotropic glutamate receptors *see* glutamate receptors



- methadone, 371  
methamphetamine, 361–2, 371  
3-methoxy-4-hydroxyphenylglycol  
(MHPG), 235  
methylphenidate, 360  
2-methyl-6(phenyleneethynyl)-  
pyridine (MPEP), 79, 80  
3-((2-methyl-1,3-thiazol-4-yl)-  
ethynyl)pyridine (MTEP),  
79, 80  
MGS-0039, 268, 268  
mianserin, 260  
microdialysis, 455  
mifepristone, 284, 285  
milnacipran, 517  
mineralocorticoid receptors, 286–7  
mismatch negativity (MNN), 27–8  
MK-0777, 143  
MK-6096, 429  
molecular weight, 482  
    multi-drug target  
        compounds, 521–2  
monoamine oxidase inhibitors  
(MAOI), 160–1, 345–6  
monoamines, 161–2  
mood disorders, 258–9  
    aetiology, 259–61  
    glutaminergic  
        neurotransmission, 261  
    metabotropic glutamate  
        receptors, 267–9  
    role of hypothalamic-pituitary-  
        adrenal axis, 278–9  
    treatments  
        anti-glucocorticoid  
            therapy, 284–6  
        arginine vasopressin (AVP)  
            antagonists, 283–4  
        CRF1 receptor  
            antagonists, 280–3  
        current schemes, 259–60  
        glucocorticoid and  
            mineralocorticoid receptor  
                activation, 286–7  
        glutamate release  
            inhibitors, 263–4  
            glutamate transporter  
                enhancers, 264–5  
            neuropeptides, 287–9  
        *see also* bipolar disorder;  
            depression  
morphine, 363  
MPEP (2-Methyl-6-  
(phenylethynyl)pyridine), 267, 268  
MRK-490, 143  
MTEP (3-((2-Methyl-4-thiazolyl)-  
ethynyl)pyridine), 267, 268  
multi-drug resistance (MDR) *see*  
    P-glycoproteins  
  
*N*-methyl-D-aspartate *see* NMDA  
NAAG (*N*-Acetylaspartylglutamic  
acid), 103  
nAChR *see* acetylcholine receptors,  
    nicotinic  
naltrexone, 370, 371  
naltrindole, 371  
narcolepsy, 416–17  
    orexin antagonists, 425–31  
    orexin mediated pathways, 421–4  
National Institute of Alcohol Abuse  
and Alcoholism, 282  
National Institute of Mental  
Health, 123–4  
NBI-30775, 281, 281, 303, 304–58  
NBI-34041, 281, 303, 305  
NBI-77860, 303, 305  
NBQX, 365  
nefazodine, 193  
nelivaptan, 284, 308  
neuregulin 1, 30  
neurexin-neuroigin  
    complexes, 391–2  
neurofibromin, 394  
neuroimaging studies, 236–8  
    anxiety disorders, 232–3  
    bipolar disorder, 335  
    challenges, 568–9  
    schizophrenia, 25–8  
    *see also* magnetic resonance  
        imaging; positron emission  
        tomography

- neurokinin receptors, 476, 477
  - multi-ligand, 529–30
- neurokinin-1 (NK1), 288, 362–3, 529–30
- neurontin, 352
- neuropathology, schizophrenia, 25–8
- neuropeptides, 287–8
  - CRF *see* corticotrophin-releasing factor
  - $\kappa$  opioid receptors, 314–16
  - MCH1, 311–14
  - neuropeptide Y, 241, 245, 289–90, 317–19
  - receptors, 321
  - regulating reward activity, 310–14
  - substance P, 241, 288–9, 362
- nevilaptan, 307, 308, 308, 309
- NEWMEDS project, 549
- NFPS, 60–4
- NGD-4715, 312
- nicotine, 125, 125, 359, 368–9, 371
  - replacement therapies, 373
- nicotinic acetylcholine receptors *see* acetylcholine receptors, nicotinic
- [alpha 7] nicotinic acetylcholine receptors *see* nAChR
- NMDA, 19, 262
  - receptor, 101–2
    - alcohol abuse, 367–8
    - role in fear response, 232–3
  - receptor (ant)agonists, 57–8, 265–6
    - D-serine, 65–6
    - ketamine, 173–4
    - mGlu receptors and, 81
    - see also* glycine
- NMDC (*N*-desmethyl-clozapine), 138, 138
- nor-BNI, 315
- noradrenergic system, 240–1
- norcapramin *see* desipramine
- norepinephrine, 161
- norepinephrine reuptake
  - inhibitors, 196–9, 345
  - dopamine reuptake inhibition and, 202–5
  - with serotonin and dopamine reuptake inhibition, 199–205, 344
- norquetiapine, 209
- novel object recognition (NOR)
  - assay, 117
- NPTS, 61
- NS-1738, 130, 130
- nucleus accumbens, 171, 171–2, 311
- octanol-water partition
  - coefficient, 475–6
- olanzapine, 37, 37, **38**, 48, **194**
- omega-3 fatty acids, 351
- opioid addiction, 363
  - see also* heroin
- opioid system
  - alcohol abuse, 370
  - cocaine, 371
- orbitofrontal cortex, 364–5
- orexins, 241, 245, 372
  - function, 417–20
  - ligands, 420–1
  - pathways controlling sleep architecture, 421–4
  - receptor antagonists, 425–31
    - dual OX<sub>1</sub>R/OX<sub>2</sub>R, 427–30
    - OX<sub>1</sub>R, 426–7
    - OX<sub>2</sub>R, 430–1
  - role in mood, 432–3
  - role in signalling and reward pathways, 431–2
  - signalling modulation, 424–5
- ORG-24598, 60–1, 61
- ORG-26576, **105**, 106
- outcome prediction, 545
- OX *see* orexin
- oxytocin, 401
- P-glycoprotein, **447**, 486–92
- PACAP (pituitary adenylate cyclase-activating peptide), 245
- panic disorder
  - animal studies, 234–5
  - clinical features, 228
  - genetic studies, 238–9

- panic disorder (*continued*)  
     human studies, 235–6  
     neuroimaging studies, 236–8  
     neuropathology, 233–5  
     role of amygdala, 230  
 Parkinson's disease, 208  
 paroxetine, 193, 289, 344  
 Pavlovian conditioning, 229  
 pergolide, 132, 133  
 periaqueductal gray (PAG), 234–5  
 pexacerfont, 281  
 PHA-543613, 127  
 pharmacokinetics, 468–72  
     comparison of classic and CNS, **470**  
 Phelan-McDermid syndrome, 389  
 phencyclidine, 264  
 phosphodiesterase inhibitors, 518  
 phospholipidosis, 500–1  
 picrotoxin, 114  
 pindolol, 185, 187, 189  
 plasma protein binding, 450–1  
 PNU-120596, 129, 130  
 PNU-282987, 127  
 polar surface area (PSA), 480–1  
 positron emission tomography (PET), 20–1  
     biodistribution imaging, 560–2  
     depression, 566–7  
     pharmacodynamic imaging, 563–4  
     proof-of-concept testing, 559–60  
     schizophrenia, 566–7  
     target engagement imaging, 562–3  
 post-traumatic stress disorder (PTSD), 172, 287  
     clinical features, 228–9  
     CRF1 antagonists, 283  
     epidemiology, 227  
     genetic studies, 243–4  
     neuropathology, 239–43  
     noradrenergic system, 240–1  
     role of amygdala, 230  
     role of prefrontal cortex, 231–2  
 Prader-Willi syndrome, 388  
 pre-frontal cortex (PFC), 21–2, 170–1, 244  
     role in fear extinction, 231–3  
     prednisone, 284  
     proopiomelanocortin, 319–20  
     protein kinase C (PKC), 347–50  
     psychiatric illnesses, 1  
     psychotic major depression (PMD), 285  
 PTEN hamartoma tumour syndrome, 393–4  
 publication bias, 537–8  
 1-[4-(1-pyrrolidinylcarbonyl)phenyl]-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1H-indazole, 107  
 quetiapine, 37, 37, **38**, 193, 193, **194**, 207  
 quinuclidines, 126, 128  
  
 R1211919, 304  
 rapamycin, 402  
 re-experiencing phenomena, 228  
 reboxetine, 205  
 reofecoxib, 175  
 repeated social defeat stress, 165  
 Research Domains Criteria (RDoC), 548–51  
 Rett syndrome, 395  
 reward pathways, orexin function and, 431–2  
 riluzole, 263, 264  
 rimonabant, 373  
 risperidone, 37, 37, **38**, 48, 397  
 Rocephin *see* ceftriaxone  
 RP-67580, 363  
 RU-43044, 284, 286  
  
 S33138, 44–5  
 salvinorin A, 314, 315  
 Saphris, 346  
 sarcosine, 61  
 saredutant, 301  
 SB-271046, 479  
 SB-277011, 45  
 SB-277011A, 361  
 SB-334867, 426–7, 426  
 SB-568849, 312  
 SB-649868, 426, 427, 434, 436

- SB-737050A, 141, 141  
SB-742457, 141, 141  
SCH-23390, 134  
schizophrenia, 4, 56–7, 99–100  
    aetiology, 17–18, 57  
        dopamine hypothesis, 20–2, 99–100  
        GABA, 23–4  
        glutamate hypothesis, 22–3, 57–8, 100  
        integrated biological theories, 31–2  
        neurochemical abnormalities, 19–20  
        role of DAAO, 66–7  
        serotonin, 24  
    animal models, MAM17, 545–6  
    cognitive impairment, 123–4  
    definition, 13–15  
    epidemiology, 15–16  
    genetics  
        association studies, 30–1  
        DAAO, 67  
        linkage studies, 29–30  
    heritability, 28–9  
    longitudinal course, 16  
    neurochemical abnormalities, 19–20  
    neuropathology, 24–5  
    outcomes, 16–17  
    PET imaging, 565–6  
    symptoms, 14–15  
    treatment options, 4, 18–19  
        *see also* antipsychotics  
    unmet needs, 8  
Schneider, Kurt, 14  
selective serotonin reuptake inhibitors (SSRI), 161–2, 184–6, 344, 516  
    augmentation, 195–6  
        5-HT<sub>1A</sub> agonism, 189–91  
        5-HT<sub>1A</sub> antagonism, 186–9  
        5-HT<sub>1D</sub> agonism, 191  
        5-HT<sub>2A</sub> agonism, 191  
        5-HT<sub>3</sub> agonism, 195  
        norepinephrine reuptake inhibitors, 196–8  
        limitations, 185–6  
        treatment of bipolar disorder, 343–4  
D-serine, 65  
serotonin, 24, 37–8  
    affinity of atypical antipsychotics, 38  
    receptor (ant)agonists, 39–40  
        5-HT<sub>1A</sub>, 48–9, 186–9, 189–91  
        agonists, 205–8  
        antagonists, 208–10  
        5-HT<sub>1D</sub>, 191  
        5-HT<sub>2A</sub>, 192–5  
        5-HT<sub>2C</sub>, 210–12  
        5-HT<sub>3</sub>, 195  
        5-HT<sub>6</sub>, 140–1  
        multiple ligand, 520, 523–7, 527–9  
        reuptake inhibition, 49–51, 161–2  
        role in schizophrenia, 24, 565–6  
    serotonin-norepinephrine reuptake inhibitors (SNRI), 344  
    SERT-plus combinations, 523–7  
    sertindole, 492  
    sertraline, 344  
    SHANK3 gene, 389  
    single nucleotide polymorphisms (SNP), 29  
    single-photon emission computed tomography (SPECT), 236–7, 237–8  
SKF-7734, 134, 134  
SKF-38393, 133, 134  
SKF-81297, 134, 134  
SLV-313, 48, 49  
SLV-314, 49, 50  
SNAP-7941, 312  
SNAP-94847, 312  
spirocyclic lactones, 188  
SSR-103800, 62  
SSR-125543, 303, 306  
SSR-149415 *see* nevilapтан  
SSR-149415, 301, 308, 309  
SSR-180711A, 128  
SSR-181507, 49  
SSR-504734, 62, 62–3, 62

- SSRI *see* selective serotonin reuptake inhibitors
- stress, 300–1, 307  
     animal models, 166–7
- strychnine, 64
- STX209, 400–2
- substance P, 241, 288–9, 362
- substance-related disorders, 7, 357–8  
     alcohol, 360–1  
     cocaine and  
         methamphetamine, 361–2  
     glutamergic signalling, 363–9, 364–7  
     neuropeptides, 362–3  
     other pharmacotherapies, 372–5  
     pharmacogenetics, 374–5  
     vaccines, 374  
     *see also* alcohol abuse;  
         amphetamine; cocaine; heroin;  
         methamphetamine
- suvorexant, 429, 429
- synaptic plasticity, 405
- T-226296, 312
- tachykinin receptors, 301
- tamoxifen, 348, 349, 350
- tandospirone, 206
- terfenadine, 499
- tetrabenazine, 360, 362
- tolcapone, 144, 144
- Torsades de Pointes*, 491
- transcription factor 4 (TCF4), 31
- traumatic events, 228
- trazodone, 193
- Treatment Units for Research on  
     Neurocognition and Schizophrenia  
     (TURNS), 101
- trichotillomania, 538, 552
- tricyclic antidepressants, 345
- trimipramine, 345
- tuberous sclerosis complex, 392, 393
- Tumor Necrosis Factor 1 (TNF-1),  
     174
- UKCP-110, 360
- unmet needs, 7–8
- V1b receptor, 307–9
- vaccines, 374
- validation, 542–7, **543**, 551–2
- valproate, 260, 265, 340, 341–2, 348
- valvular heart disease (VHD), 490
- Vanderbilt, 138–9
- venlafaxine, 197, 516, 517
- ventral tegmental area (VTA), 368–9,  
     433
- vesicular glutamate  
     transporters, 261–2
- vesicular monoamine transporter-2,  
     361
- vilazodone, 189, 190
- WAY-100635, 39, 40
- WAY-101405, 209–10, 209
- WAY-163909, 210, 211
- WS50030, 50
- X-ray crystallography, 107–9
- xanomeline, 137, 138, 545
- XBD-173, 244–5
- XOMA, 052, 174–5
- XY-4083, 130–1, 130
- years lost to disability (YLD), 184
- yohimbine, 235
- zimeldine, 185, 186
- ziprasidone, 37, 37, **38**, 39–40, 48,  
     193, **194**, 527–9, 528
- ZNF-804A, 31